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HORMONES AND THE ANALYSIS OF GROWTH[1]

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(WITH ELEVEN FIGURES)

It is now about twenty years since the carefully controlled experiments of PÁAL established finally that growth in plants is under the control of special growth substances or *hormones*. In the intervening period our knowledge has advanced considerably, though mainly only in respect to this one rather well-defined group of hormones, the *auxins*. These substances, as you know, have been isolated from both plant and animal sources, including both seed plants and fungi, and their chemical nature has been thoroughly elucidated. Furthermore, large numbers of synthetic substances have been prepared which have similar actions. Their chemistry will be discussed further below; it is enough for the moment to note that they have rather clearly defined similarities.

The physiology of these growth substances, however, shows much more diversity. The action studied by BOYSEN-JENSEN, PÁAL, SÖDING, WENT, and the other workers up until the last few years was an action upon cell enlargement, causing growth in the simplest sense of the word. The hormone was envisaged as the mechanism by which the influence of the tip of the plant was exerted on the growth of the part below it. However, it was not long before evidence was forthcoming that other quite different influences of one part of the plant upon another were brought about through the action of the same hormones.

The first instance of this was the *inhibition* of one bud by another. It had been previously indicated that this well-defined inhibiting action was perhaps due to a special inhibiting substance, and we were able to show not only that it is, indeed, but also that the substance is identical with auxin. Thus a substance which promotes growth in some tissues also characteristically inhibits it in others.

A more marked instance of the same thing is seen in roots. Here all but

[1] Fifth STEPHEN HALES address, read at the Indianapolis meeting, December 28, 1937.

the most extremely low concentrations of auxins inhibit elongation, so that root tissue, like bud tissue, is readily inhibited by auxin. However, it has recently been found both in our own and other laboratories that *excessively* low concentrations, of the order of 1 gram in 10 million liters, actually accelerate growth of roots. These are concentrations of almost unbelievable lowness. GEIGER has calculated that to dilute a gram of indole-acetic acid so that it is completely inactive on *Zea mays* roots would take an amount of water such that if it had to be brought by rail, it would require 400,000 trains, each of 50 ten-ton wagons. Such trains would comfortably extend around the world, or a quarter of the way to the moon.

This phenomenon of growth acceleration and growth inhibition is probably paralleled in all other plant material. Generally the inhibiting concentrations are much higher than in roots, but the difference is apparently quantitative rather than qualitative. The relation is something like that shown in figure 1.

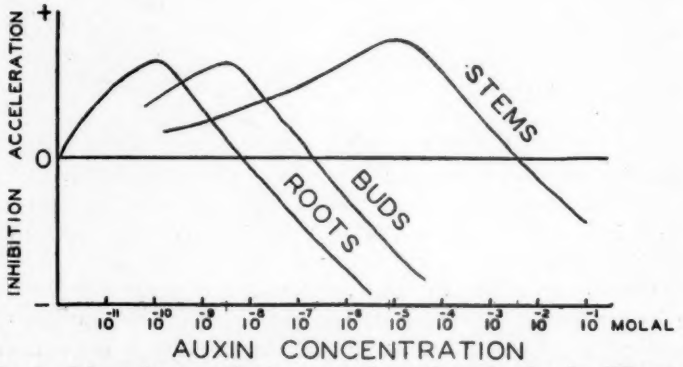

FIG. 1. Scheme for approximate ranges of growth acceleration for different plant organs.

The inhibition of roots involves a very interesting *after-effect* which we have recently been studying, and which is a good example of one type of the analysis of growth. In this case an observed, rather ill-defined acceleration of growth can be traced back to the initial, much slighter acceleration of growth which caused it. If roots are treated with auxin so that they are inhibited, and the auxin afterwards removed, their subsequent growth is accelerated. They catch up with the untreated controls and even surpass them. Figure 2 shows this effect with *Avena* roots. *Triticum* behaves similarly. The higher the concentration of auxin used, the longer it takes for this acceleration to appear, but on the whole the greater the acceleration is when it does come. Thus the roots treated with lowest auxin concentrations are the first to pass the controls, and those treated with highest concen-

trations are the last, but even these plants treated with highest concentrations, whose roots scarcely grow at all during the treatment, afterwards have roots considerably longer than the controls.

At the same time, the very high auxin concentrations also increase the *number* of roots (at least in seedlings of certain plants). In *Avena* the treated seedlings may develop 20 or more roots as against 5 to 7 in controls of the same age (fig. 3). The result of both these responses is that the treated plants, although at first inhibited, subsequently have a far better root system. The roots are both longer and more numerous. As a result the development of the shoot is accelerated, since water supply is exceedingly

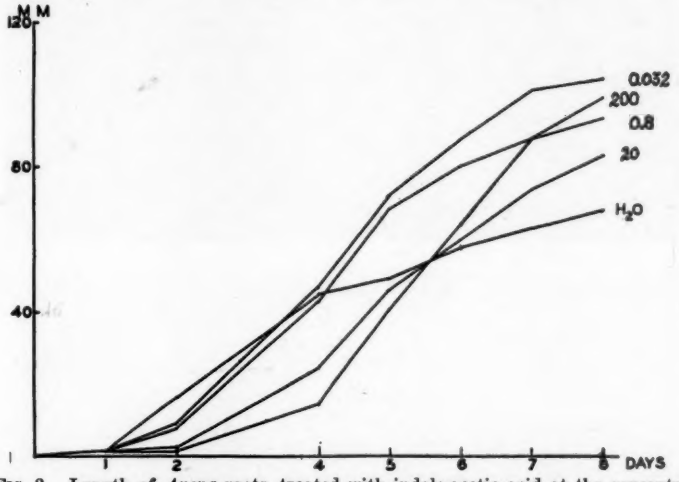

FIG. 2. Length of *Avena* roots, treated with indole-acetic acid at the concentrations marked (in mg. per liter) from the first to the second day of growth and then transferred to water.

important in the growth of young plants. This acceleration of the shoot can be very marked. Figure 4 shows such treated *Avena* plants, after 5 weeks in the cool greenhouse, with controls. Only the plants treated with highest auxin concentration (100 mg. per liter) are shown. The others behaved intermediately. Not only is there a general acceleration, but the leaves are broader and hence the photosynthetic area is increased. This leads to huskier plants. The formation of broader leaves has been obtained in other plant material also.

Thus we have here a growth acceleration resulting weeks later than the initial treatment, involving a number of changes such as increase in height, growth rate and leaf area, which can be analyzed back, with a high degree of probability, to the initial effect on the root system of the seedling. This

FIG. 3. Right, two *Avena* seedlings treated with indole-acetic acid. Left, control in water.

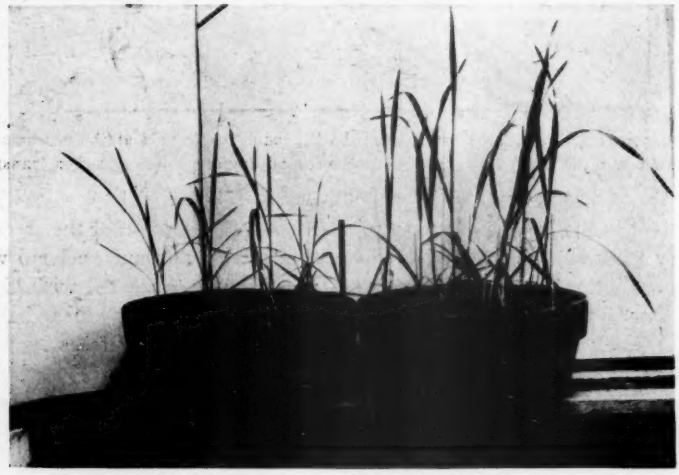

FIG. 4. Right, *Avena* plants grown from seedlings treated during first and second day of growth and transferred to water. Left, controls treated only with water. Five weeks old.

FIG. 5. Concord grape cuttings (one-year wood). Below, treated with indole-acetic acid, 200 mg. per liter for 24 hours at base. Above, controls treated with water. Photographed after 14 days in unheated sand.

shows how complex the analysis of growth phenomena can be, particularly when they are long-term effects.

Now, as you know, another action of the auxins, and it is common, in varying degree, to all the different substances which are auxins, is the induction of root formation. Like other auxin effects this is common to the majority of plants. Figure 5 shows the effect of twenty-four hours' treatment with indoleacetic acid on the rooting of Concord grape cuttings. Many similar photographs have been published recently for a variety of plants. In the normal plant, root-formation is achieved by the migration, to the base, of auxin produced in the growing buds and in the leaves, the transport which obtains normally in the plant being polar. This polarity of the transport of auxin brings about the formation of roots predominantly at the *bases* of cuttings, just as it is also responsible for the influencing of growth by buds or other auxin-forming organs below rather than above them. Of course, if we apply auxin to the plant externally, this polarity of its transport may be obscured by the upward and outward movement to which auxin (like salts or any other externally applied material) is subject. Nevertheless in the intact plant it is evident that there is a rather strict polarity of auxin transport, and the fact that under the right experimental conditions it can be demonstrated and even measured is important for the analysis of growth problems.

The first stages of root development involve rapid cell division and the formation of a root initial. Since this is brought about by auxin, it is perhaps not surprising to find that cell division in other tissues is also activated by auxin. This is mainly true only for the cambium. In tissues other than the cambium, however, cell division can be stimulated, though usually only by unphysiologically high auxin concentrations such as are produced by artificial application, or by the invasion of pathogenic micro-organisms. There does not, therefore, seem to be so clear a distinction between the factors causing division and those causing enlargement as was formerly thought. The influence of auxin in causing the development of parthenocarpic fruits, recently demonstrated by GUSTAFSON, must be considered in the same connection. It is of interest also that the wound hormone, recently studied by BONNER and ENGLISH on the pericarp of the bean, may cause either enlargement alone or enlargement accompanied by cell division according to the variety of bean used as test object. Evidently here also the factors causing the two processes are somewhat interlocked.

We are faced, therefore, with the fact that this one group of hormones, the auxins, bring about a variety of processes of growth and inhibition, and the effect that they exert evidently depends on the kind of cell they enter and on its physiological state. In the earlier days of this work, some four to five years ago, when only the action on cell enlargement was recognized,

it was customary to consider that the auxin causes growth by combining with the cell wall in some way. However, we were able to show that there is no stoichiometrical relation whatever between the auxin that enters the cell and any of the constituents of the cell walls. Further, this variety of physiological functions obviously indicates that the auxin action is exerted upon some very fundamental process in the cell, some kind of "master reaction." The action has been aptly compared to the unlocking of a door; once unlocked, the doorway no longer controls who or what shall pass in or out; these depend upon the conditions on either side of the door. However, the problem of the unlocking of the door becomes more interesting and important than ever because so fundamental. Similar problems of physiology are associated with the action of all hormones and vitamins, and in no case is there a really clear understanding of their actual mode of action.

There have been two main lines of approach to this unlocking problem; one consists in the analysis of the *key, i.e.,* the determination of just what properties the hormones must have in order to act—the other is the analysis of the *lock, i.e.,* an attempt to determine more nearly the nature of the master reaction and the other processes brought about by auxin. I will discuss each of these briefly.

Let us consider first the key. The formulae of figure 6 show that the

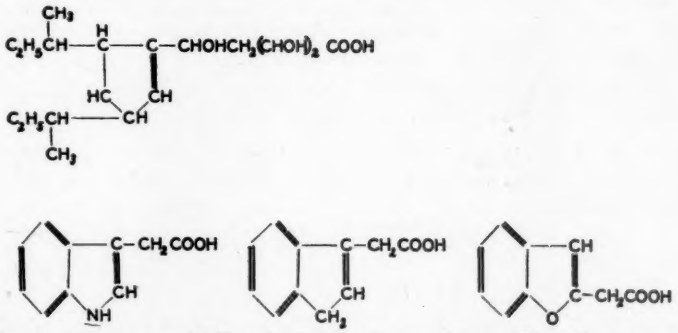

FIG. 6. Above, auxentriolic acid (auxin a) according to KÖGL, HAAGENSMIT, and ERXLEBEN. Below, left to right, indole-3-acetic acid, indene-3-acetic acid, benzofurane-2-acetic acid.

substances auxin a and indole-acetic acid, the two most highly active auxins, both have an unsaturated 5-membered ring with an acid group in the side-chain. One has a nitrogen atom, the other not. The first subject for study was, therefore, the activity of comparable compounds without the nitrogen atom. Both these substances (formulae to the right) were found to be active, though much less so than indole-acetic acid. A very interesting difference lay, however, in their behavior in the *Avena* test, in which the auxin

is applied in agar to one side of the coleoptile. In this test, indole-acetic acid produces curvature distributed down the coleoptile for 15 mm. or so, but indene-acetic acid, the compound with carbon instead of nitrogen in the ring, produces only localized curvatures (fig. 7). To produce curvature there

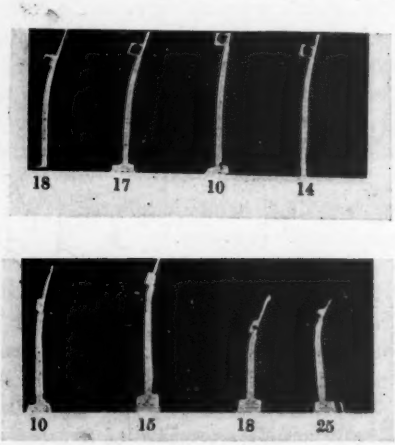

FIG. 7. Decapitated *Avena* seedlings supplied with auxin in agar on one side. Above, indole-3-acetic acid, curvature distributed. Below, indene-3-acetic acid, curvature localized.

must be a marked differential growth and hence the substance must be restricted to the side to which it is applied. If it does not move far on that side it can cause only local curvature—if it spreads out equally on to the other side it can cause no curvature at all. The physiological concentrations of indene-acetic acid evidently do not move as rapidly through the tissues as indole-acetic acid, *i.e.*, their transport is poorer, and this is supported by the fact that indene-acetic acid causes excellent rooting of cuttings if applied at the base, *i.e.*, at the point where its action will occur, but little or no rooting if applied at the tip, from whence it will have to move to the base to bring about its effect.

The second difficulty, that of spreading out, is shown by the oxygen derivative, cumaryl-acetic acid, or benzofurane-acetic acid, which causes straight growth but no curvature. A later analysis of the 3-derivative, benzofurane-3-acetic acid has shown it to be essentially similar though a little more active. Hence here we are dealing with properties which are not essential for *growth* activity, but which control the way in which the growth comes to be manifested; in this case they influence the ability of the substances to be linearly transported.

For further analysis we have therefore tried to study growth activity in

such a way that transport does not enter in. For this purpose immersed plant parts, such as the slit pea stems whose curavature was shown by WENT to be so convenient a tool for auxin work, have been used. Figure 8 shows

FIG. 8. Curvatures of slit internodes of etiolated pea stems. Left to right, water, 0.1, 1.0 and 4.0 mg. per liter indene-acetic acid.

single young internodes of etiolated *Pisum*, slit longitudinally and immersed in water or auxin solutions. The stem halves, which curve slightly outwards in water, curve inwards in auxin solution on account of differential response to auxin. The inward curvature varies roughly with the logarithm of the auxin concentration. Being immersed, the auxin is applied all round and transport difficulties need not concern us, except insofar as the rates of entry might be different at the different surfaces. One or two interesting points as to the relation between structure and activity have been brought out in this work. First as to the need for the double bond in the auxin molecule. KÖGL found that the saturation of the double bond in indole-acetic acid and auxin a caused complete loss of activity (fig. 9). The same thing is shown by phenyl-acetic acid (active) and cyclohexyl-acetic acid (inactive). Cyclohexene-acetic acid, with one double bond, on the other hand, is active. If the double bond is in the sidechain and not in the ring, as in cyclohexylidene-acetic acid, the activity is again lost, hence not only must the double bond be present, but it must be in the ring. Up to now all open-chain compounds have been found inactive.

Secondly as to the structural arrangement (fig. 10). The acid group cannot be next to the ring, since indole-carboxylic and benzoic acids are inactive. Apparently it must be free to reach a certain position in space which is about one carbon atom distance from the ring. Introduction of one methyl group in the side chain does not affect this orientation, but the two methyl groups on the same place prevent it from doing so; to a lesser degree it is prevented from reaching this position by being farther out on the chain, for, in general, activity decreases with increasing length of the sidechain, (phenyl-acetic > β phenyl-propionic > γ phenyl-butyric). A special case is

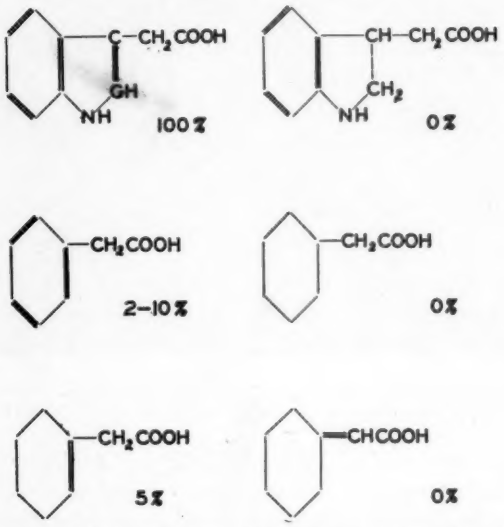

FIG. 9. Top, left, indole-acetic acid; right, dihydro-indole-acetic acid. Middle, left, phenyl-acetic acid; right, cyclohexane-acetic acid. Bottom, left, cyclohexene-acetic acid; right, cyclohexylidene-acetic acid.

that in which the double bond in the sidechain prevents free rotation; *cis*-cinnamic, in which the approximate position can be reached, is active, while *trans*-cinnamic, in which the COOH is held out at arm's length, so to say, has no activity. The same holds for derivatives of *cis* and *trans*-cinnamic acids. It is evident that within this group of substances the requirements for activity are quite strict, and the action is therefore rather *specific*. When this is more clearly understood we shall be in a position to formulate possible reactions in which the auxin may take part and begin to find out which one occurs.

The other approach is by analysis of the lock. In this, an important piece of evidence is the fact that auxin acts in the tissues together with other sub-

stances. Of these, the best understood is sugar. In order for auxin to cause growth in the coleoptiles, sugar is essential. If the sugar supply is first exhausted from the plant, auxin can cause no growth. If auxin alone is supplied, sugar greatly increases the growth. Not only sugar, however, but other factors are also concerned. This is particularly true in root formation

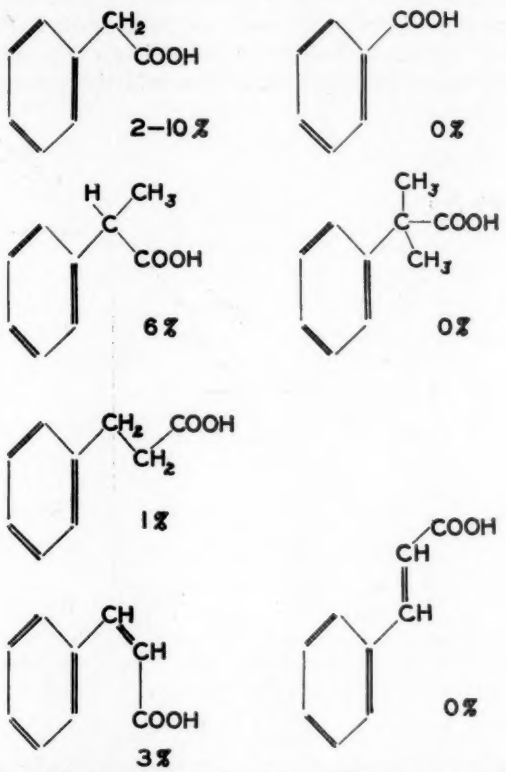

FIG. 10. Top, left, phenyl-acetic acid; right, benzoic acid. 2nd row left, α-methyl-α-toluic acid; right, αα-dimethyl-α-toluic acid. 3rd row, β-phenyl-propionic acid. 4th row, left, *Cis*-cinnamic acid; right, *trans*-cinnamic acid.

on cuttings (fig. 11). Here, in *Pisum* cuttings, sugar alone makes about two roots per plant, probably using the auxin already there. Holding the sugar constant and optimal, the number of roots increases proportional to the *auxin* concentration, to a maximum of about 10 roots per plant. Then, holding the auxin constant and maximal, we can increase the next factor (biotin, yeast

growth factor) to reach a new maximum. When this is held at the maximum value, there is evidence for a fourth factor.

Thus the analysis of the lock must take into account the other parts of the lock besides the key. WENT has suggested that these other factors, or certain of them, are really the substances which are specific for the different processes, special substances in the sense of SACHS. A better understanding of the rôle of these other factors will depend on further experiments.

Lastly, as to the nature of the first reaction into which the auxin enters. We now have excellent evidence that it is an oxidation process. I will not

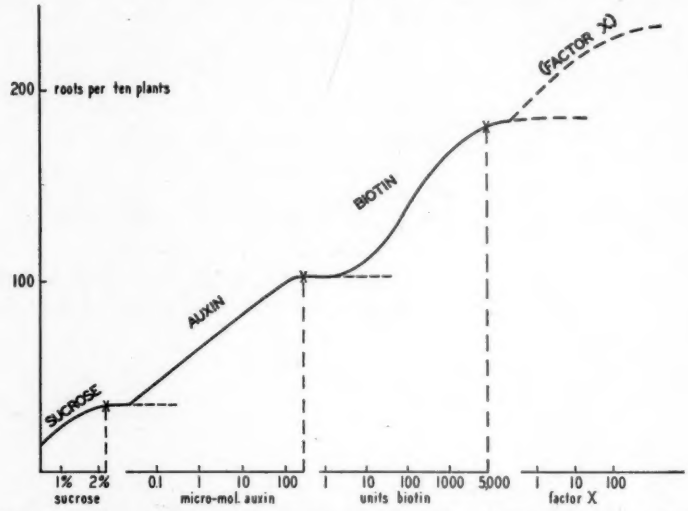

FIG. 11. Interaction of factors causing root formation in *Pisum* cuttings. Ordinate, roots per 10 plants; abscissa, concentration (log. scale) of the factor which is limiting root formation. (From WENT and THIMANN, Phytohormones. New York, Macmillan. 1937).

discuss this in detail now, but will only say that when auxin is applied to the cell there is an immediate acceleration of the rate of protoplasmic streaming. This acceleration is observed much sooner than any growth can be detected. It is like growth in that it needs sugar for its effect to be fully realized. It also needs oxygen; in absence of oxygen, streaming is retarded; and in auxin, the consumption of oxygen by this reaction is increased. Hence auxin probably has as its first effect an increase in the rate of one special oxidation process, a process whose substrate is sugar, and which directly controls the rate of streaming, and, probably through this, the rate of growth. The streaming of protoplasm in the cell is something so fundamental that it is easy to see how changes in its rate can bring about a variety of subsequent

changes. It is not yet known whether we can say that this reaction which controls streaming and growth is the primary door which auxin unlocks; if not, it is very near it.

In conclusion I would like to say two things about this analysis of growth problems through hormones. In the first place, it must be remembered that it is plant *physiology*, not *pharmacology*, *i.e.*, we do not, as some people imagine, simply apply all kinds of substances externally to plants, but we are engaged in the study of processes which go on in the normal plant.

Secondly, it is not purely academic. One immediate result of the work has been as you know, the elucidation of the action of auxins in causing root formation on cuttings, a finding of immediate practical utility. In this respect it would have been strongly approved by STEPHEN HALES, who, more than 200 years ago, pointed out that the only sound basis, both of agriculture and horticulture, must be plant physiology; (I quote from his vol. 1): "As the art of Physick has of late years been much improved by a greater knowledge of the animal œconomy, so doubtless a farther insight into the vegetable œconomy must needs proportionably improve our skill in Agriculture and Gardening, which gives me reason to hope that enquiries of this kind will be acceptable to many, who are intent upon improving those innocent, delightful and beneficial Arts: Since they cannot be insensible that the most rational ground for Success in this laudable pursuit must arise from a greater insight into the nature of Plants."

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EXANTHEMA IN PEAR, AND COPPER DEFICIENCY

JACOB OSERKOWSKY AND HAROLD E. THOMAS

(WITH FOUR FIGURES)

Introduction

The present paper deals with a disease of Bartlett pear trees which occurred in two orchards in the coastal region of central California (Contra Costa County). In one orchard the affected trees were scattered, somewhat in groups, over an area of several acres. The majority of these trees were not seriously injured. In the other orchard, several miles distant from the first one, most of the sick trees were severely injured, and occasionally one of them died during the period of observation in 1930 to 1934. The diseased trees were confined in this orchard to an area of about 20 to 25 acres. Insofar as the appearance and growth of the trees was concerned, there was a more or less sharp line of demarcation between the diseased and the healthy areas. Most of the trees in the diseased part of the orchard were suffering from the malady. The various treatments and analytical data concerning the affected trees to be discussed in the following sections refer to trees in the diseased area of this orchard. The disease was diagnosed by SMITH and THOMAS (8) as *exanthema,* and it will be referred to in this paper as such, although this does not imply that the disease is necessarily identical in its causes with other plant disorders designated by the same name.

DESCRIPTION OF THE DISEASE

The symptoms of the disease are as follows: During the latter part of May or early June the extreme tips of the current-year shoots turn brown and die; almost simultaneously the tips of the terminal shoot leaves also turn brown and die. The dying of the shoots and leaves progresses downwards with the season (fig. 1), and in severely affected trees three-fourths or more of the current year's growth may be dead by the end of the summer. Dead leaves usually fall to the ground leaving the denuded shoots on the tree. This process is repeated year after year and occasionally a tree degenerates to the point of death. As a result of the death of the apical growing points, shoot growth from axillary buds is stimulated, in consequence of which trees affected with exanthema present a semi-witches'-broom appearance and become rather dense and bushy (fig. 3). A limited development of bark excrescences results in the bark on older branches becoming somewhat more roughened than on trees unaffected with exanthema. The formation of gum has not been observed. Affected trees bear practically no fruit.

Perhaps the most characteristic symptom of exanthema in pear is the

appearance of the affected leaves. The dying of the leaves progresses from the tip and the margin toward the center (fig. 2); the dead portion exhibits alternate brown-orange striation running parallel with the outline of the leaf. Not all affected leaves show this striation, but its occurrence in abundance was observed only on pear trees affected with exanthema. In this respect the symptoms of the disease in pear differ from those in other trees (**4, 8**). Chlorosis as a symptom of exanthema in pear could not be observed

FIG. 1. Pear shoots of the current season exhibiting various degrees of exanthema symptoms. Right, healthy shoot; extreme left, shoot severely affected with exanthema.

by the writers. The green portion of leaves severely "burnt" along the margin may be somewhat faded but the leaves are never typically chlorotic. The symptoms of exanthema are unlike those of any known parasitic disease of the pear.

That the disease is associated with the soil upon which these treees grow can be inferred from the fact that young seedlings of Bartlett pear, the seeds of which were taken from a region free of the disease and grown in that region in pots containing soil from the affected orchard, exhibited, after a few months, symptoms of exanthema.

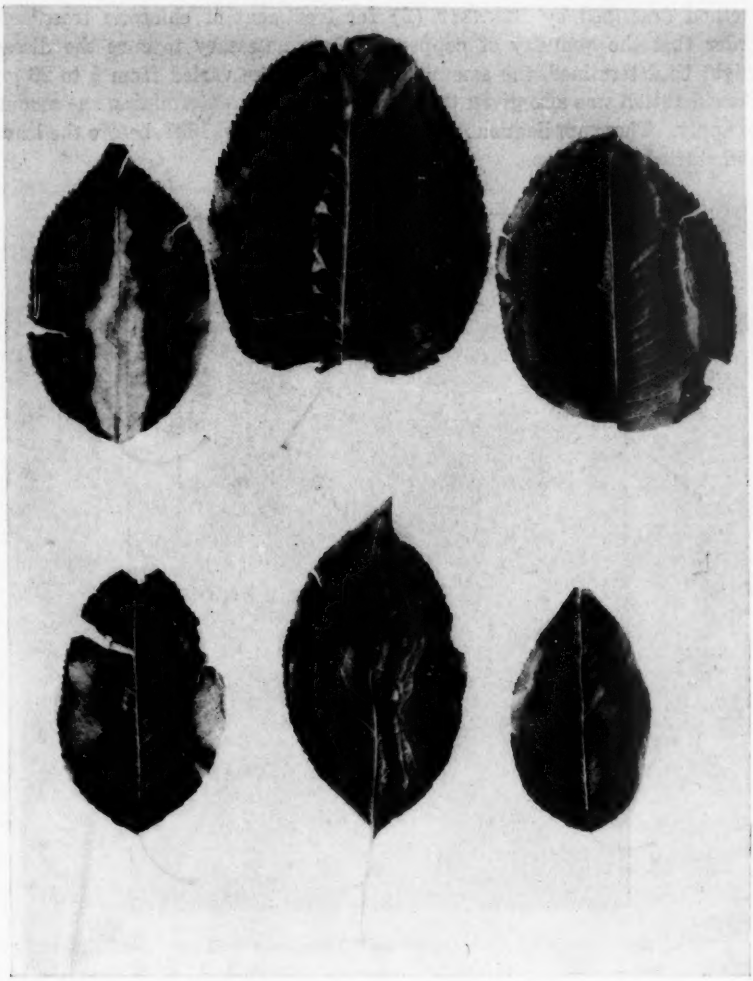

FIG. 2. Pear leaves from exanthema trees, exhibiting tip and marginal burning with striations.

Experiments

I. EFFECT OF COPPER ON EXANTHEMA

Copper sulphate crystals were introduced into trees suffering from exanthema, in the lower part of the trunk near the root crown, using the

method described by BENNETT (2) for treatment of chlorotic trees.[1] In order that the quantity of copper sulphate necessary to cure the disease might be determined, the amount applied per tree varied from ¼ to 20 gm. Consideration was also given the size of the tree in determining the amount to apply. These applications were made in February, 1931, before the leaves had started to develop.

FIG. 3. Pear tree injured by exanthema. Terminal shoots have dead tips and are partially denuded. Numerous leaves are marginally "burnt."

Since the orchard had not been charted for the occurrence of exanthema previous to these treatments, the exact condition of the trees at the time of treatment was somewhat uncertain. In many, however, evidences of the disease were present, even after a severe pruning, and such trees were selected for the application of the copper salts. If the trees had not been

[1] Preliminary accounts of these treatments were published by THOMAS (10) and by OSERKOWSKY and THOMAS (7).

TABLE I

EFFECT OF INTRODUCTION OF $CuSO_4 \cdot 5H_2O$ CRYSTALS INTO TRUNK OF PEAR TREES SUFFERING FROM EXANTHEMA

TREATMENT OF TREES	AMOUNT APPLIED	No. OF TREES	CONDITION* OF TREES IN SUMMER														
			1931					1932					1933				
			0	+	++	+++	++++	0	+	++	+++	++++	0	+	++	+++	++++
			IN NUMBER AND PERCENTAGE OF TOTAL TREES IN EACH GROUP														
Trees treated with $CuSO_4 \cdot 5H_2O$ in February, 1931	*gm.* 0.25	3	3	0	0	0	0	2	1	0	0	0	2	1	0	0	0
	0.5	4	1	1	1	1	0	2	1	1	0	0	0	4	0	0	0
	1.0	7	1	3	2	1	0	2	4	1	0	0	1	3	2	1	0
	2.0	11	6	3	2	0	0	8	3	0	0	0	7	4	0	0	0
	3.0	3	2	1	0	0	0	1	2	0	0	0	3	0	0	0	0
	4.0	5	4	1	0	0	0	4	1	0	0	0	3	2	0	0	0
	6.0	2	2	0	0	0	0	2	0	0	0	0	2	0	0	0	0
	8.0	2	1	1	0	0	0	2	0	0	0	0	2	0	0	0	0
	10.0	4	2	1	0	1	0	4	0	0	0	0	4	0	0	0	0
	15.0	2	2	0	0	0	0	2	0	0	0	0	2	0	0	0	0
	20.0	2	2	0	0	0	0	2	0	0	0	0	2	0	0	0	0
Total no.	.....	45	26	11	5	3	0	31	12	2	0	0	28	14	2	1	0
Percentage of total	.....	.....	58	24	11	7	0	69	27	4	0	0	62	31	4	2	0
Trees affected with exanthema in 1931 but not treated with copper salts																	
Total no.	0	140	0	12	31	49	48	3	32	36	39	30	6	28	38	47	21
Percentage of total	.....	.....	0	9	22	35	34	2	23	26	28	21	4	20	27	34	15

* Symptoms of exanthema: 0 = healthy in appearance, no symptoms; + = slight; ++ = moderate; +++ = moderately severe; ++++ = severe.

treated it is safe to assume that most of them would have exhibited symptoms of exanthema during the summer of 1931. The trees were treated in alternate rows in a block of the orchard severely affected by exanthema. In the summer of 1931 and in each of the two subsequent summers a chart was made of this part of the orchard describing the condition of the trees. The trees were divided into two groups, namely, those treated with copper sulphate and the trees not treated with copper sulphate, which in the summer of 1931 exhibited exanthema. The results are summarized in table I. It is apparent that copper sulphate in small doses cured the trees partially or completely. Further, the beneficial action of copper sulphate lasted over three seasons. The diseased branches on some of the treated trees may be due to an inadequate supply or distribution of copper from the holes in the root crown. Such is also the case in lime-induced chlorosis when the holes containing the iron salt are not properly distributed around the trunk.

Pear trees suffering from exanthema can be cured also by other copper salts. Thus, 7 of 8 diseased trees treated with $CuCl_2$ during the dormant season showed very marked improvement during the following growing season. Similar response was obtained with $Cu(NO_3)_2$. The less water-soluble copper salts also exert a beneficial action on diseased pear trees, although the latter respond more slowly, and one or two seasons may elapse before the action of these salts becomes apparent. This is illustrated in table II. The doses ranged from 1 to 9 gm. of copper salts per tree. The trees were treated in February, 1931.

TABLE II

EFFECT OF INTRODUCTION OF CRYSTALS OF SPARINGLY SOLUBLE COPPER SALTS INTO TRUNK OF PEAR TREES SUFFERING FROM EXANTHEMA. TREES TREATED FEBRUARY, 1931

No. of tree	No. of row	Tree treated with	Condition* of trees in summer		
			1931	1932	1933
36	1	Copper carbonate	+++	+	+
38	3	" "	+++	+	tr.
40	4	" "	+++	tr.	0
36	2	Copper phosphate	++	+	+
38	2	" "	tr.	0	0
40	6	" "	+++	+	tr.
36	6	Copper tartrate	++++	++	++
38	5	" "	++	+	0
40	5	" "	+++	+	+

* Notations same as those of table I. Tr. = trace.

Bordeaux spray also exerts a beneficial action (*cf.*, figs. 3 and 4). Branches on diseased trees sprayed with it early in the spring exhibit vigorous growth and are free of the symptoms of exanthema during the remainder of the season. The action of Bordeaux spray is localized; only the sprayed

branches show recovery from exanthema. The effect of this spray lasts only one season.

$CuSO_4 \cdot 5H_2O$ crystals spread around diseased trees within a radius of a few feet from the trunk and incorporated in the top soil exerted only a slow action on exanthema. Their effect was not apparent until 2 to 3 years after the treatment. Small doses of the copper salt often resulted in slight improvement only. This is not surprising in view of the fact that the soil in the treated orchard was very heavy, with presumably a large capacity for fixing Cu. Further, the yearly precipitation in the region where the orchard is situated is only about 20 inches. Irrigation is not practiced. As a result of this the $CuSO_4 \cdot 5H_2O$ dissolves very slowly. Thus, several large crystals of this salt were found on the surface of the soil, and in the uppermost layer of the soil around a treated tree, two years after the application. Table III

FIG. 4. Pear tree sprayed in the spring with Bordeaux mixture. Originally showed severe exanthema symptoms. Compare with figure 3.

TABLE III

EFFECT OF APPLICATION OF CRYSTALS OF $CuSO_4 \cdot 5H_2O$ TO TOP SOIL AROUND PEAR TREES SUFFERING FROM EXANTHEMA

TREE	ROW	DATE OF APPLICATION OF $CuSO_4 \cdot 5H_2O$ TO SOIL	AMOUNT OF $CuSO_4 \cdot 5H_2O$ APPLIED TO EACH TREE	CONDITION* OF TREES IN SUMMER		
				1931	1932	1933
			lb.			
3	3	February, 1930	5	++++	+	++
6	3	" "	5	++	+	tr.
8	3	" "	5	+++	tr.	0
7	7	" "	2½	++	++	+
8	7	" "	2½	++++	++	+
16	7	February, 1931	50	+++	0	0
16	5	" "	30	+++	++	tr.
16	4	" "	20	+++	+++	+
38	12	" "	20	++++	++	0
38	13	" "	10	++++	+	+
14	5	" "	10	++++	0	tr.
44	12	" "	5	++++	+++	0

* Notations same as those of table I. Tr. = trace.

presents the results obtained with applications of $CuSO_4 \cdot 5H_2O$ crystals around trees suffering from exanthema.

Affected trees were treated also with salts of various metallic elements. The salt crystals were introduced during the winter into holes bored in the trunk of trees a few inches above the root crown. The method of application was that used by BENNETT (2) for treatment of chlorotic trees. Four or five trees were treated with each salt, and the doses used (equivalent to one or a few grams of the metal per tree) were similar to those of $CuSO_4$, where treatment was beneficial. The following salts were used: $MnCl_2$, $ZnSO_4$, ferric citrate, $K_2Cr_2(SO_4)_3$, $NaVO_3$, $Co(NO_3)_2$, and $Ni(NO_3)_2$. None of these treatments attenuated exanthema, although in many instances the foliage and twigs were badly injured, indicating that the salts reached the upper parts of the treated trees and were fairly well distributed.

It is thus clear that application of copper salts, whether to the soil in the form of salt crystals, as Bordeaux spray to the leaves, or as crystals introduced into the trunks of trees, resulted in complete recovery of the trees, or at least in a marked reduction in severity of exanthema symptoms and very noticeable increase in growth. Further, copper in small doses (1 to 2 gm. per tree weighing several scores of kilograms) exerted a *specific* beneficial action on trees affected with exanthema, whereas salts of other heavy elements were without action on the disease. This led to the supposition that exanthema may be due to a deficiency of copper. In order to test this hypothesis, analysis of pear tissues from healthy and diseased trees was undertaken. The results of these analyses are discussed in the following sections.

TABLE IV

COPPER CONTENT OF BARK AND WOOD FROM HEALTHY AND DISEASED PEAR TREES

DESCRIPTION OF SAMPLE	DATE COLLECTED	HEALTHY TREES FROM REGION FREE OF EXANTHEMA		HEALTHY TREES FROM A HEALTHY BLOCK IN AFFECTED ORCHARD		HEALTHY TREES FROM SAME BLOCK AS AFFECTED TREES		AFFECTED TREES		HEALTHY TREES AS RESULT OF COPPER TREATMENT	
		BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD
		MG. CU PER KG. OF DRY WEIGHT									
Terminal part of shoot	May 12	*mg.* 12.2		*mg.* 4.9		*mg.*		*mg.*		*mg.*	
	May 12			4.6							
	May 30	.....		.....		.....		3.2		.....	
	June 1	.....		.....		4.5		2.7		14.2	
Basal part of shoot	June 1	.....	.....	.....	.....	4.1	3.6	3.5	4.6	16.6	11.6
Terminal part of shoot	July 16	11.8	11.4	6.5	4.4	.....	.....	4.2	2.7	5.5	3.2
Basal part of shoot	July 16	9.0	7.3	7.4	4.0	.....	.....	3.7	1.9	3.7	3.0
One year or older growth	July 16	12.4	5.9	5.6	2.3	.....	.....	3.0	1.2	4.7	1.8

II. Copper contents of healthy and diseased tissues

Shoots and branches were collected from healthy and diseased trees stripped of their leaves and analyzed for copper. The data are presented in table IV. The samples collected in May were too young to be separated into basal and terminal shoots. In this instance the sample consisted of the upper six inches only, and the bark was not separated from the wood. In subsequent collections the upper six inches of the current-year growth was designated as "terminal part of shoot" while the remaining part, approximately of the same length, was termed "basal part of shoot." Shoots and branches were collected from the affected orchard and from healthy trees in a locality free of the disease (Berkeley). The analytical data show that the copper content was highest in trees grown in the locality free of the disease and in trees in the diseased block which, in consequence of treatment with copper sulphate, recovered from exanthema. The copper content of all samples from trees in the healthy part of the affected orchard was higher than the corresponding values for the samples from the diseased trees. There was, however, no significant difference between the amount of copper in the trees suffering from exanthema and the trees healthy in appearance in the affected part of the orchard. A similar relation holds true also in the case of leaves (table V) and will be discussed below.

Pear leaves were collected from various localities at different times dur-

TABLE V

Copper content of pear leaves from healthy and diseased trees

Group of samples	Type of leaves	Locality collected	No. of samples	Range of Cu content in p.p.m. of dry weight
				p.p.m.
A	Healthy leaves	Regions free of exanthema	40	10 –41
B	Healthy leaves	Three regions free of exanthema within ½ to 6 miles from affected orchard	6	12 –17.5
C	Healthy leaves	Healthy block in affected orchard, from 20 to 100 yards from diseased trees	10	4.9– 7.6
D	Healthy leaves	Healthy trees in affected block of orchard	4	4.5– 5.6
E	Healthy leaves from trees suffering from exanthema	Affected block of orchard	11	3.7– 6.7
F	Leaves affected with exanthema	Affected block of orchard	6	3.2– 5.1

ing the growing season and from healthy and diseased trees in the affected orchard and in orchards adjacent to it. The results are given in table V. The *A*-samples were collected from three orchards situated in two regions about 25 and 80 miles, respectively, from the orchard affected with exanthema. Some of the samples are of Hardy pear; the majority, however, are of Bartlett pear trees, the same variety as the affected trees. The inclusion of the Hardy samples in this table has very little effect upon the conclusion to be drawn, since there were no significant differences in copper content between samples of the two varieties. The copper content of the samples from the two localities was also practically identical.

The *B*-samples were obtained from three orchards from ½ to 6 miles from the affected orchard.

The *C*-samples were gathered from trees healthy in appearance, and of normal vigor, grown in the affected orchard, but on the edge of the affected block. These trees were 20 to 100 yards from the sick trees.

The *D*-samples were obtained from a few trees healthy in appearance scattered in the affected block of the orchard. Although these trees were free of symptoms of exanthema, they made, for the most part, a rather subnormal growth, and thus may be considered as being on the verge of exhibiting exanthema.

The data in table V strikingly show that the copper content of leaves from regions free of exanthema is decidedly higher than that of either healthy or diseased leaves from the affected orchard. The copper content of the former is never below 10 p.p.m. while the highest recorded copper content of the latter was not above 7.6 p.p.m. and even this value is for healthy trees outside the affected portion of the orchard. The average was about 4 p.p.m. There is, however, no significant difference between healthy and diseased leaves from the area affected with exanthema. This fact is not incompatible with the assumption that exanthema is due to copper deficiency. A similar situation is obtained in the case of lime-induced chlorosis curable by administration of iron salts. It was shown in that case that not all of the iron in leaves is active in chlorophyll formation, that only a portion of the iron participates in chlorophyll formation, and this fraction was termed "active iron." Chlorotic leaves are poorer in active iron than green leaves from chlorotic trees, although the iron content of the latter might be below that of the chlorotic leaves (**6**). Similarly it may be assumed that not all of the copper in leaves is active (whatever that activity may be), and that leaves affected with exanthema contain less active copper than healthy leaves in the affected orchard, although there need be no significant difference in the total copper content of these two kinds of leaves.

III. Copper content of basal and terminal shoot leaves

The leaves first to show symptoms of exanthema are the terminal shoot leaves (fig. 1), and the dying of leaves progresses downward. It was expected therefore that the copper content of terminal shoot leaves from sick trees, whether they exhibit symptoms of exanthema or whether they are about to exhibit them (*e.g.*, collected early in the season), would be smaller than that of basal leaves from the same trees. This, however, was not the case, as is readily evident from the data in table VI. Although the terminal leaves, healthy in appearance, are somewhat higher in copper content than the corresponding terminal leaves affected with exanthema, the difference is small and of doubtful significance. In any event the copper content of the affected terminal leaves was *higher* than that of the basal shoot leaves which when collected did not exhibit symptoms of exanthema, although this difference also may not be significant.

The samples collected on July 16, 1931, were obtained at a time of the season when the dying of leaves on exanthema-affected trees had progressed far, while on May 12, 1933, visible injuries to the leaves were just making their appearance. On this date five composite samples of terminal and basal shoots, respectively, were collected (table VI, columns 4, 5). The "healthy

TABLE VI

Copper content of basal and terminal shoot leaves from diseased trees

Description of leaves	Collected 7/16/31		Collected 5/12/33	
	Cu content in p.p.m. of dry wt.	$\frac{Cu}{N} 10^4$	Cu content in p.p.m. of dry wt.	
			Sample 1	Sample 2
	p.p.m.		*p.p.m.*	*p.p.m.*
Terminal shoot leaves affected with exanthema	4.0	1.6	4.5	.....
Healthy terminal shoot leaves from trees affected with exanthema	4.5	1.9	5.0	6.7
Healthy basal shoot leaves from trees affected with exanthema	3.6	1.5	3.5	3.4
Healthy spur leaves from trees affected with exanthema	4.2	1.6	.....	.....

terminal shoot leaves" collected on this date from the sick trees may represent leaves on the verge of exhibiting symptoms of exanthema, since it is very likely that if left on the trees for a few weeks longer many of them would have shown the typical symptoms of exanthema. In any event, such terminal

leaves would have begun dying earlier than the basal leaves on the same shoots, and yet the copper content of these terminal leaves was *higher* than that of the basal leaves.

It was thought that the terminal leaves being younger were richer in protoplasm and therefore required for normal functioning larger amounts of copper per unit of dry weight. Since there is no satisfactory measure of the amount of protoplasm in plant tissues, the nitrogen content of the leaves was used as an approximate, although admittedly crude, measure of the protoplasmic content of leaves. The ratio of Cu to N is reported in column 3 of table VI for the samples collected on July 16, 1931. This ratio may be considered as a very rough measure of the ratio of copper to protoplasm. It is seen, however, that the relation thus obtained between healthy and affected terminal and basal shoot leaves is substantially the same as when the copper content is reported on the dry weight basis.

IV. Copper content of marginal and central part of leaves

Since the injury to leaves affected with exanthema manifests itself in progressive dying of the leaves from the tip and margin toward the center, it was reasonable to expect that the central part of the leaf would contain more copper than the marginal and apical part. Composite samples were obtained from sick trees at various times. The margin and tips of the leaves were severed from the central part with scissors. Only one sample consisted of leaves with dead tips. Analysis showed that the central part of this leaf sample contained 2.3 p.p.m. copper, and the dead margin and tip 2.2 p.p.m. This is an insignificant difference. Several more samples of terminal and basal shoot leaves were collected from sick trees, but the leaves of these samples were free of dead margins or tips. If left longer on the trees many of them no doubt would have exhibited symptoms of exanthema. It is permissible therefore to characterize the condition of these leaves as being in the state of incipient exanthema. In this case also, contrary to expectation,

TABLE VII

Comparison between copper content of apical and marginal, and central part of pear leaves

Date of collection	Terminal shoot leaves		Basal shoot leaves	
	Central part of leaves	Apical and marginal part of leaves	Central part of leaves	Apical and marginal part of leaves
	Cu content in p.p.m. of dry weight			
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
5/31/33	1.7	3.0	1.8	1.7
5/31/33	1.7	2.6	2.0	2.1
6/5/33	.....	.....	1.9	1.9

there was no consistent difference in copper content between the marginal and the central parts of the leaves (table VII).

Discussion

Exanthema and copper deficiency

Unlike deficiency of iron in higher plants, which, as far as it is known invariably manifests itself as chlorosis, copper deficiency is characterized by a multitude of symptoms. Thus, Sommer (9) observed that sunflowers, tomatoes, and flax grown in a culture medium deficient in copper made very little growth compared with the check plants adequately supplied with this element. On the other hand, Lipman and Mackinney (5) found no significant difference in dry weight of barley plants raised in a culture solution deficient in copper, when compared with the check plants. In this instance, however, the barley deficient in copper developed fewer flowers, and produced practically no seeds. Thus in barley, it appears, deficiency of copper resulted in curtailment of the reproductive organs, and hindered normal development of the embryo. Haas and Quayle (4) studied a case of exanthema in citrus which could be cured by copper salts, and which they succeeded in producing by withholding copper from plants grown in pure silica sand and irrigated with a culture solution. This malady must be thus regarded as due to copper deficiency, yet the symptoms are quite different from those recorded by Sommer, and Lipman and Mackinney for annual plants. Anderssen (1) described a chlorosis attributed to a deficiency of copper and affecting some deciduous fruit trees. Pear trees growing in the same region did not exhibit chlorosis but were suffering from a die-back which, as far as could be ascertained from the description of the injury, was similar to the symptom of exanthema on pears. Anderssen does not mention, however, the striated bands on injured leaves, neither does he specifically refer to the response of affected pear trees to treatment with copper compounds. Sufficient data are lacking to ascertain whether the disorder of pear trees reported by Anderssen is identical with the exanthema described herein.

It is thus evident that none of the aforementioned descriptions of the various manifestations of injury to higher plants caused by copper deficiency exactly tallies with the symptoms of exanthema on pear. The literature on this subject does not offer, therefore, a criterion for ascertaining whether or not exanthema is due to a copper deficiency. On the other hand, the marked response of pear trees affected with exanthema to various treatments with copper salts in small doses, the specific effect of copper salts, and the low copper content of plant tissues in the affected orchard bespeak in favor of the hypothesis that exanthema in pear trees is due to a copper deficiency. Crucial evidence in support of this view, however, is lacking. The inducement of exanthema in pear seedlings grown in culture solution in the

absence of copper, and its subsequent cure by administration of salts of this metal could be considered as such evidence. It was attempted therefore to grow Bartlett seedlings in a solution free of copper. Unfortunately the plants used in this experiment were injured, probably by excessive amounts of the rarer elements like Pt, Pb, Co, Ni, and Cd which were added to the culture medium.

Since exanthema is produced by some soil factors it might not be illogical to assume that the disorder is brought about by soil microorganisms which either directly attack the roots or else produce harmful substances. Accordingly the effect of copper would be either to kill or inactivate these microorganisms or to neutralize the effect of the toxic compounds produced by them. The first assumption, however, is untenable, since pear shoots on sick trees respond readily to Bordeaux spray, yet in this case curative action of the spray is strictly localized and confined to the sprayed shoots, thus proving that the copper of the spray had not been translocated within the plant. And yet, despite the fact that it did not reach the roots or the root zone it exerted a very beneficial effect. The supposition that exanthema is due to microorganic toxins absorbed by the roots and transferred to the apical parts of the plant seems improbable in view of the specific curative effect of copper and the absence of any beneficial action from other heavy metals like Co, Ni, Cd, Mn, and Zn. There exists the possibility that microorganisms in the soil could interfere with the absorption of copper by the tree roots. For the reasons given and pending evidence to the contrary, the writers prefer to consider exanthema as due to copper deficiency *per se*. This view may account for most of the correlations existing between copper and exanthema, but it does not furnish a simple explanation for the type and progress of the injury as discussed in sections III and IV, unless recourse is had to the hypothesis that terminal leaves and the margin of leaves contain less active copper than basal leaves or the central portion of leaves respectively; or else that the former require larger amounts of the active copper compound for normal functioning.

Analytical details

All plant material was thoroughly washed in distilled water, and rinsed with it several times prior to drying, excepting two leaf samples obtained in the orchard affected with exanthema. The analytical data obtained with these samples were the same as those secured with non-washed leaves.

Copper was determined colorimetrically essentially according to the method described by Elvehjem and Lindow (3). During the course of the work several minor changes were introduced in the analytical procedure. With the material on hand there was no need to separate iron from copper.

With each set of samples one or two blanks were run, and the copper

values for the samples were corrected for them. The copper content of the samples varied from about 8 to 100 micrograms (μ. gm.). It was found that Baer's law holds true with sufficient accuracy for our purpose within at least the following limits of concentration: 14 to 96 μ. gm. of Cu dissolved in 10 cc. of chloroform as a green pyridine-thiocyanate compound. All determinations, with the exception of a few, were carried out in duplicate or triplicate. The average accuracy of the analytical data is about 5 per cent.; it is somewhat lower in the case of the samples with the smaller copper contents.

Summary

1. Exanthema on Bartlett pear trees in central California is described. This disease is attributed to soil conditions. Trees suffering from it can be cured by application of copper salts to the soil, by introducing crystals of copper salts into the root crown of the tree, or by spraying affected trees with Bordeaux mixture. Copper salts exert a *specific* beneficial action on trees suffering from exanthema since $MnCl_2$, $ZnSO_4$, ferric citrate, $K_2Cr_2(SO_4)_3$, $NaVO_3$, $CdSO_4$, $Co(NO_3)_2$, and $Ni(NO_3)_2$ do not correct exanthema.

2. The copper content of leaves and shoots from trees within the area affected with exanthema is invariably lower than that from samples obtained in localities free of the disease. There is, however, no consistent difference in the copper content of healthy and diseased trees in the orchard affected with exanthema. A similar relation exists with regard to iron in chlorotic trees.

3. Exanthema in all probability is due to a deficiency of copper *per se*.

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WATER RELATIONS IN *BRYOPHYLLUM CALYCINUM* SUBJECTED TO SEVERE DRYING

WALTER BURCHARD WELCH

(WITH TWO FIGURES)

Introduction

Bryophyllum calycinum Salisb. will reproduce vegetatively by means of plantlets formed in the notches of the vegetative leaves. This reproduction, called regeneration by LOEB (**9**), has been rather thoroughly treated by LOEB (**10**), REED (**17**), BRAUN (**1**), CHILD and BELLAMY (**2**), and others. A great deal of attention has been paid by these authors to the reproduction of the young plants, but little attention has been given the water relations in the parent leaf during their formation and growth.

In most of the work reported the leaves, when removed from the plant, were placed in contact with water or at least in a moist atmosphere. Under laboratory conditions, without extraneous water, the plantlets appear on the margin of all portions of the leaf without regard to polarity (fig. 1, A, B). The leaves, when hung up by their petioles, produce young plants on the basal half as well as on the apical half, but when placed with one edge in water or in moist soil the shoots will appear only where the leaf is in contact with the water or moist soil.

The parent leaf may dry until it becomes brittle at its central portion, yet the young plants along the margin will still be turgid and continue to increase in size. Since this leaf is capable of living for a long period and reproducing new tissues without additional water being supplied to it, it was proposed that a study be made of the water relations of the parent leaves and their plantlets. A great number of phenomena might be studied in connection with the ability of these leaves to withstand severe drying: osmotic concentration of the juices of the leaves and plantlets, electrical conductivity of these juices, hydration of the ions present in the solutions, relationship of the freezable and unfreezable water to the total water, the thickness of the cuticle, the distribution of stomata, and so on. The work presented in this paper will deal with the freezable and unfreezable water in their relation to the total water, the thickness of the cuticle, and the distribution of stomata.

Unfreezable water has attracted a great deal of attention among plant physiologists who were interested in cold resistance or the ability of plants to become hardened against killing at low temperatures. ROSA (**19**) found that in cabbage the rate of decrease in the percentage of freezable water during hardening coincided with the rate of hardening of plants against killing by low temperatures. GREATHOUSE and STUART (**6**) found that

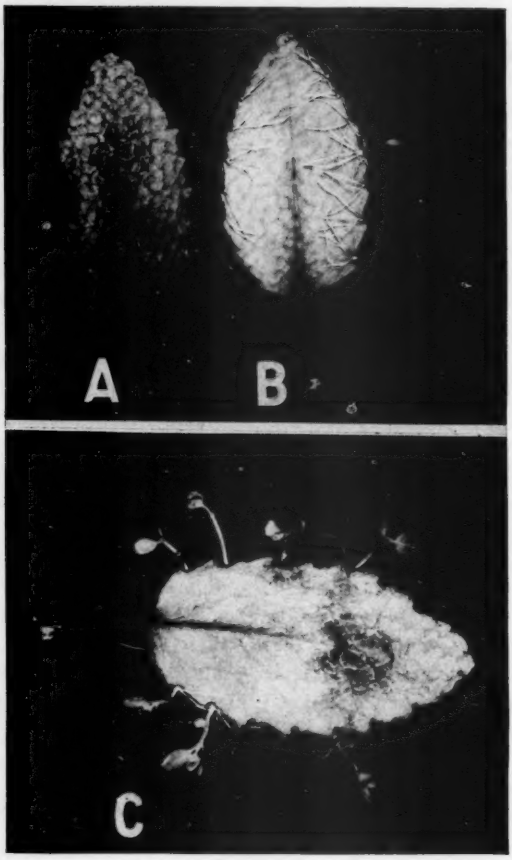

FIG. 1. A. Upper surface of leaf of *Bryophyllum calycinum* after 4 weeks of drying, showing plantlets in notches of leaf (½ natural size). B. Lower surface of leaf showing maximum production of roots at 4 weeks (½ natural size). C. Upper surface of leaf in A showing plantlets and drying central region after 6 weeks (⅔ natural size).

Ohio and French varieties of red clover could be differentiated as to cold hardiness by the percentage of unfreezable water in the fresh tissues. STARK (**21**) demonstrated that in general the capacity of an apple twig to retain water against freezing is associated with winter hardiness. He could not, however, grade the hardy and tender varieties in any ascending or descending order on the basis of their ability to retain water in the unfrozen state. In *Pinus rigida* there was only about 5 per cent. more water in the

leaves in the summer than in the winter, and MEYER (13) states that there is no evidence that "bound" water plays any rôle in cold resistance in this species. He suggested "that the basis of cold resistance lies in some as yet not understood, physio-chemical properties of the protoplasm, which probably cannot be discovered by the gross measurements which are generally employed at present." DUNN (3) found that *Bryophyllum*, subjected to a temperature of −1.1° C., would temporarily build up a resistance to this cold temperature to such an extent that a certain number of plants would not be killed after exposure for 15 hours. Surviving plants were propagated for six generations by leaf cuttings. There was a rise in the number of survivors after three generations of cuttings but after six generations there was a return to the original number.

From these and other reports it seems possible that there is a positive correlation between unfreezable water and cold resistance in some species or varieties; but in other species or varieties such a positive correlation between unfreezable water and cold resistance may not be demonstrable.

The terms "free" and "bound" water, used by the earlier investigators, no longer convey the idea investigators wish to express. It is quite possible that under a given set of conditions and at a given time, all or nearly all of the water in a plant may be "bound," and under other conditions and at another time nearly all of the water may be "free." It would be better to refer to the "bound" as unfreezable water and the "free" as freezable water. It then becomes necessary to define the conditions under which the freezing, in the heat of fusion method of determination of freezable water, is carried out. The water is held in the tissues, cells, or parts of cells against freezing in equilibrium with the surrounding atmosphere, or any other tissues, cells, or parts of cells with which they may come in contact. This has been pointed out by GREATHOUSE (5) in his work on red clover roots and tissues of the potato tuber. He found that there was a greater amount of water frozen in the tissues at −50° C. than at −22° C. JONES and GORTNER (7) found that as much water was frozen out of gelatin at −6° C. as was frozen out at −50° C. They suggested that plant tissues should behave as did the gelatin, but the experiences of GREATHOUSE show that there is a freezable-unfreezable water equilibrium that has a different value for every temperature used.

In this work on *Bryophyllum* the temperature of −20° C. was chosen as the one at which the freezable-unfreezable water equilibrium should be measured. There is nothing to suggest that −50° C. would be the lower limit at which the freezable water can be measured; any obtainable temperature above absolute zero might be chosen for such measurements. Since an equilibrium relation is being dealt with, the most important feature is the maintenance of the selected freezing temperature for sufficient time to

permit an equilibrium to become established, and to hold that temperature constant within a range of $\pm$ 0.25° C.

The problem of drought resistance or of resistance to very severe drying in relation to the unfreezable water content of the tissues has received much less attention than the relation of unfreezable water to cold resistance. VASSILIEV and VASSILIEV (**23**) found that in general the most conspicuous feature in which a wheat plant, that had been hardened to resist drought, differs from a normal one is in the greater accumulation of hemicelluloses and sugars, chiefly sucrose. The plants which had been exposed to drought by withholding water for 18 days, but were later given an ample water supply, were found to be deficient in water after 8 days of irrigation. They were, however, higher in most forms of carbohydrates except the monosaccharides. This water-deficient, high-carbohydrate condition became more or less "fixed" in plants that had recovered from the drought conditions.

The concentration, osmotic pressure, and "bound" water of the juices of some of the cultivated and some of the wild species of grasses, were found by NEWTON and MARTIN (**16**) to increase with the progress of maturity. The unfreezable water was found to increase markedly more rapidly than the osmotic pressure and appeared to be more stable. LEBEDINCEV (**8**) used unfreezable water measurements to determine the water-retaining capacity of xerophytes and mesophytes growing under xerophytic conditions in a semidesert region. She has shown that xerophytes are distinguished by having a higher water-retaining capacity than mesophytes under the same conditions. If the plants were subjected to repeated wiltings there was an increase in the water-retaining capacity which was maintained by the plants after recovery from wilting.

Bryophyllum is usually not considered a xerophyte unless MAXIMOV's (**11**) criterion, "the main peculiarity of xerophytes is the capacity of enduring permanent wilting without harm or without harm to their subsequent development," is used. The "wilting without harm to their subsequent development" is true only of leaves that are removed from the plant while they are in a turgid condition. If the leaves become dry on the plant and then fall or are removed, they will not produce plantlets on the margin even though roots may have been present at the notches of the leaves while they were still on the plant.

The series of determinations reported in this paper were undertaken to throw light, if possible, on the significance of the unfreezable water in both the parent leaf and the regenerating tissues, and the significance of any shift or redistribution of total water between parent leaf and its plantlets, with special reference to severe drying of the parent leaf and the ability of its regenerating offspring to persist and develop after the parent leaf had partially dried.

Materials and methods

The *Bryophyllum calycinum* used was that which MEHRLICH (**12**) has called the Chicago variety. This variety has been grown in the University of Chicago greenhouses for a number of years. Although it has been observed to flower no seeds have been obtained. The plants were vigorously grown under greenhouse conditions until they had produced eight sets of leaves, then the leaves from the fourth and fifth nodes were removed. In preliminary tests, these leaves from the fourth and fifth nodes were found to produce more shoots per leaf than the leaves from any other node. The leaves were allowed to dry in screen-wire trays on a laboratory table for as much as 18 weeks. After a week, the leaf became limp; after 2 weeks, roots appeared at the notches; and after 4 weeks the shoots of the plantlets appeared (fig. 1). The development of the roots and shoots has been adequately described by NAYLOR (**15**) and YARBROUGH (**24**). The leaves, after removal from the plant, did not come in contact with any extraneous water except that of the laboratory atmosphere.

The leaf continued to dry until at 10 weeks the central portion, indicated by the shaded area in figure 2, had become tough and brown while the tissues along the edge and base became even more turgid and remained green. The solid line in figure 2 indicates the manner in which the leaf was cut to separate that portion enclosed by the line, hereafter called the central region, from that portion outside, hereafter called the edge and base. This latter portion includes the petiole in all cases.

The method used to determine the unfreezable water was that of RUBNER (**20**) as modified by ROBINSON (**18**). Most of the equipment was that described by ROBINSON. The cold chamber was well insulated and deep enough that a minimum change in temperature at the level of the material was observed when the lid was removed. The temperature was maintained at $-20°$ C. $\pm$ 0.25. Two thermopiles were made of no. 30 gauge double-silk insulated copper and constantan wire, on which two coats of shellac had been brushed. The thermopile used in the cold chamber to check the temperature was one of only five junctions, while the one used in the calorimeter had twenty junctions and produced a potential of 763 microvolts per degree difference in temperature. The "cold" junction in each case was in a thermos bottle filled with a mixture of crushed ice and ice water. The potential in microvolts was read from a Leeds and Northrup type K potentiometer.

The calorimeter was a highly evacuated, highly silvered Dewar flask, 12 mm. in diameter and 65 mm. deep. The flask was sealed into a three-fourths-inch layer of cork with sealing wax at either end, and finally wrapped in tape and varnished. This insulation made it practically impossible to touch the edge of the calorimeter with the fingers during the transfer of the

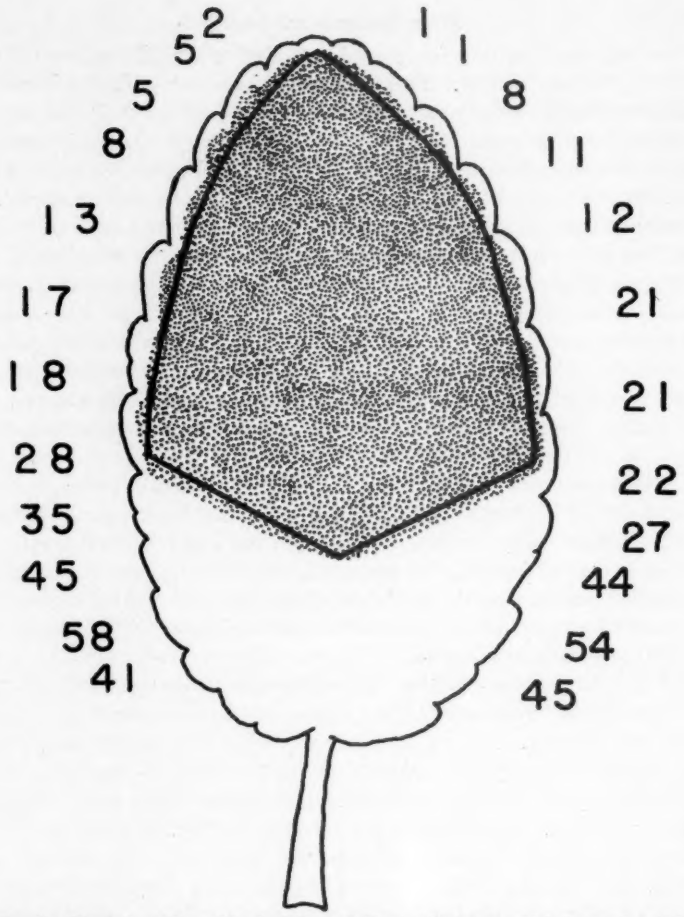

FIG. 2. Shaded portion of leaf of *Bryophyllum calycinum*, which is tough and brown, showing extent of drying at 10 weeks. Solid line shows how leaf was cut to separate central portion from the edge and base. Numbers at margin represent the plantlets counted on 100 leaves varying in age from 8 to 12 weeks.

material from the cold chamber to the calorimeter. The calorimeter was placed in a water bath maintained at 30° C.

A spiral glass stirrer was used to mix the water in the calorimeter. To the stirrer motor a flexible cable was attached which was held in place by two glass bearings in ring stand clamps. The lower end of the cable was attached to a steel bearing which could be fastened rigidly in place. The

glass stirrer fitted into a chuck on the steel bearing shaft. The motor and bearings were so mounted on a hollow rod that the stirrer could be swung in and out of the calorimeter in minimum time; and when not in the calorimeter, the stirrer dipped into a flask of distilled water in the water bath. Owing to the heating of the motor and the bearings, factors from 1.196 to 1.226 had to be used for the thermal capacity of the calorimeter in a continuous series of ten determinations. Never more than ten determinations were made in any one series.

The freezable water was calculated by use of the formula presented by ROBINSON (**18**):

$$X = \frac{FN(T_2 - T_3) - (WSR + W_1S_1R)}{80 - \frac{T_1}{2}}$$

in which F = correction factor for the thermal capacity of the calorimeter
N = volume of water in cc. used in calorimeter (10 cc.)
T_2 = initial temperature of water in calorimeter
T_3 = final temperature of water in calorimeter
W = total weight of the material
S = specific heat of the material
R = range of temperature between T_1 and T_3
W_1 = weight of tin-foil cup
S_1 = specific heat of tin foil which is 0.05
T_1 = initial temperature of material in cold chamber

This being below zero appears to have a minus sign but the formula is so constructed that the sign may be disregarded.

X = freezable water

The unfreezable water is found by subtracting the freezable water from the total water present. The specific heat of each specimen was calculated from the specific heat of the dry material, which was found to be 0.0871, and the specific heat of water, 1.

The plant material was chopped with a razor blade and ground to a paste in a mortar. This thoroughly ground material was then placed in a tin-foil cup in a numbered vial and stoppered. The samples ranged in weight from 0.25 to 0.85 gm., but the usual sample weighed about 0.5 gm. After freezing, the cups were removed from the vial and the tops bent down in such a manner as to form waterproof balls, after which they were replaced in the cold chamber and frozen for at least one hour.

The vial was removed from the cold chamber by grasping the stopper with the fingers of the right hand. The upper end of the vial was held with the fingers of the left hand only long enough to remove the stopper and slide the specimen into the calorimeter. After thawing, the material was dried in a vacuum oven at 47° C., using a pressure of 0.04 atm.

Results

Determinations of freezable and unfreezable water

The data were obtained from three series of experiments. The first series was performed to determine the distribution of the total, freezable, and unfreezable water in the whole plant. This information was necessary not only to establish the normal relationship in the leaves and stem, but also to determine how the leaves at the fourth and fifth nodes compared with the other leaves.

The second series was designed to determine the relationship of total, freezable, and unfreezable water in the whole leaf, with its attached plantlets, during the period of drying. The data from this series were to serve as a guide for the third series of measurements.

In the third series the purpose was to determine the relationship of the water, as in the second series, in the different parts of the leaf and the attached plantlets. In this latter series of measurements it was thought that some light might be thrown on the relation between the freezable and unfreezable water in the parent leaf and in the young plants with special reference to any shift or redistribution of water there may be between the two during the severe drying.

Series 1. Distribution of total, freezable, and unfreezable water in the whole plant

The plants used in this series of experiments were vigorously growing individuals that had nine sets of leaves below the apical bud, and a portion of the stem below the ninth set of leaves that was bare. It was impossible to separate the apical bud from the first internode and the first set of leaves and obtain a specimen that would be comparable in weight to the other samples of the stem internodes and the leaves. Therefore the apical bud, first internode, and the first set of leaves were chopped, ground, and frozen as a single sample. The second set of leaves was then removed and treated in the same manner; then the second internode, and so on down the stem.

The lowermost portion of the stem was made up of six very short internodes without leaves, and was treated as a single sample. The determinations on this portion of the stem were entered in table I as the basal internodes. This lower portion of the stem was very woody and rather difficult to reduce to the same degree of fineness as the other samples.

As may be seen from the data of column 2, table I, the first six internodes contained more total water and more freezable water than was found in the leaves attached at the first six nodes, when calculated on the dry weight basis. Internode three contained the largest amount of water of any part examined, and the basal internodes the least. From internode three to internode eight

there was a gradual decrease in total water from 10.385 gm. of water per gram of dry weight to 2.692 gm., and on the same basis the freezable water decreased from 9.706 to 2.368 gm. of water (column 3, table I).

The second and third internodes (column 5, table I), contained a smaller percentage of unfreezable water, 5.62 and 6.53 per cent., respectively, than the leaves at the second and third nodes, 10.40 and 8.66 per cent. The internode contained 7.65 per cent. of unfreezable water while the leaves at the fourth node had only 5.69 per cent. This relation, the higher percentage of unfreezable water in the stem than in the leaves attached to that portion of the stem, was observed in all parts examined, below the fourth node. That portion of the stem referred to as the basal internodes had only 12.05 per cent. of unfreezable water while the seventh internode had 12.66 per cent. This decrease may possibly be due to experimental error, but it may also indicate a reversal of the water relationship in the older stem regions.

The third internode contained more total water per gram of dry weight than any other part of the plant (column 2, table I) and, therefore, less dry weight. There is a smaller percentage of unfreezable water in the third internode than in any of the other internodes. As the stem decreases in total and freezable water, the percentage of unfreezable water increases; or, if the unfreezable water were calculated on a fresh weight basis, it would increase as the dry weight increases. The bud, with the first set of leaves and the first internode, contained more total and freezable water than any of the leaves except the pair at the eighth node. This exception was noted in all plants examined. The eighth pair of leaves appear to be water storage organs. There was a greater percentage, 9.11, of unfreezable water in the bud than in any of the leaves except those at the seventh node which had 9.66 per cent. of unfreezable water. This increased amount of unfreezable water in the leaves at the seventh node is not accounted for, and was seen in all plants examined. The leaves at the fourth and fifth nodes had an average amount of total, freezable, and unfreezable water and were chosen for use in series 2 and 3.

The data in table I are averages of four determinations on three plants where sufficient material was available.

Series 2. Determination of total, freezable, and unfreezable water in the whole leaf

In this series, the whole leaf with its attached plantlets, was treated as a unit, to determine, if possible, the effect of drying on the amount of total, freezable, and unfreezable water. Leaves from the fourth and fifth nodes were placed in screen wire trays and allowed to dry from 1 to 13 weeks. The drying of the leaf was first observable in the central region along the midrib; it then proceeded toward the edge, until at 10 weeks the central

region of the upper portion of the leaf was tough and brown. The edge and base remained turgid and green (fig. 2). The leaves, with attached plantlets, were chopped, ground, and frozen in the manner already described.

TABLE I

TOTAL, FREEZABLE, AND UNFREEZABLE WATER IN LEAVES AND INTERNODES OF *Bryophyllum* EXPRESSED IN GRAMS PER GRAM OF DRY WEIGHT, AND THE PERCENTAGE UNFREEZABLE WATER

MATERIALS	TOTAL WATER IN GM./GM. DRY WT.	FREEZABLE WATER IN GM./GM. DRY WT.	UNFREEZABLE WATER IN GM./GM. DRY WT.	UNFREEZABLE WATER IN PER CENT.
	gm.	*gm.*	*gm.*	*%*
Bud with first two leaves and internode	8.447	7.678	0.769	9.11
2nd leaves	7.770	6.691	0.808	10.40
2nd internode	10.233	9.657	0.575	5.62
3rd leaves	7.355	6.718	0.637	8.66
3rd internode	10.385	9.706	0.678	6.53
4th leaves	6.470	6.101	0.368	5.69
4th internode	9.446	8.723	0.723	7.65
5th leaves	5.395	5.018	0.376	6.98
5th internode	8.367	7.643	0.723	8.64
6th leaves	4.818	4.614	0.204	4.23
6th internode	7.189	6.431	0.766	10.64
7th leaves	6.409	5.789	0.619	9.66
7th internode	3.751	3.276	0.475	12.66
8th leaves	9.471	8.776	0.694	7.33
Basal internodes	2.692	2.368	0.324	12.05

During the first 2 weeks of drying the leaves became limp and lost 16.54 per cent. of their total water. There was a greater amount of total water, 9.026 gm./gm. dry weight, after 3 weeks of drying than in fresh leaves, 7.719 gm. (column 2, table II). During the third week the roots in the notches of the leaves grew more vigorously than at any other time (fig. 1, B). During the next 8 weeks there was from 18 to 58 per cent. more water in the drying leaf than in the fresh leaf on the dry weight basis. It was during this period that shoots of the regenerating plantlets exhibited the most active growth (fig. 1, C). After 11 weeks the plantlets did not noticeably increase in size.

Since the leaf had no access to water, except that present as vapor in the atmosphere, it is evident that internal changes were causing a reduction in the dry weight. This might be expected, since the energy and materials for the production and growth of the plantlets must be derived from the parent leaf. If there were any increase in internal solids as a result of photosynthetic activity in the leaf or the plantlet, it could not have been measured except as a difference between the material produced and that used by the growing plantlets.

There was a greater percentage of unfreezable water in the leaf and young plants after drying for 13 weeks than in fresh leaves (column 5, table II). There was no gradual increase in the unfreezable water as drying progressed, as might have been expected, but rather a decrease from 8.09 per cent. in the fresh leaf to 3.41 per cent. in the fifth week. The percentage of unfreezable water does not appear to increase or decrease in exact proportion to the total or freezable water, or to the dry weight. It only roughly varies directly with the dry weight, and therefore inversely to the freezable water. Thus any equilibrium that is established between the freezable and unfreezable water must vary from day to day independently of the drying process.

It will be noted that during the eighth week there was less total water than during the seventh and ninth weeks (column 2, table II). The roots had completed their growth during the fifth week and the most vigorous growth of the shoots of the young plants took place during the ninth and tenth weeks. The percentage of unfreezable water in leaves that had dried for 8 weeks was higher than at any time from the second to the thirteenth week (column 5, table II). After 11 weeks the leaves dried out rapidly having less freezable and total water, and in the thirteenth week more unfreezable water in gm./gm. dry weight. This rapid drying is probably the factor that caused the plantlets to stop their growth. They could maintain themselves but could not increase in size on the amount of water they could obtain from the parent leaf.

The data in table II are averages of five determinations on each of three leaves, in all of which, if attached, the plantlets were considered a part of the leaf.

Series 3. Determination of total, freezable, and unfreezable water in parts of the leaf and plantlets, during drying

In series 3 determinations were made on the different parts of the leaf, and on the plantlets produced at the notches of the leaf to determine the amount of total, freezable, and unfreezable water in each portion and the changes in the quantitative relation to the drying process. Since the leaves first dry out in the central region and leave a margin of green turgid tissue at the edge and base, the leaf was so sectioned that the central region was examined separately from the edge and base. The leaves that had not dried more than 6 weeks were cut along a vein leading to the fourth notch from the base of the leaf; then along the margin, one-quarter of an inch from the edge, to the apex. After 6 weeks, the leaf was limp and yellow (fig. 1, C), in the central region with a definite line of demarcation between the dry area and the turgid area at the edge and base. Then the leaf was cut, as shown in figure 2, along the solid line.

TABLE II

TOTAL, FREEZABLE, AND UNFREEZABLE WATER IN GRAMS PER GRAM OF DRY WEIGHT, AND UNFREEZABLE WATER IN PERCENTAGES

MATERIAL	TOTAL WATER IN GM./GM. DRY WT.	FREEZABLE WATER IN GM./GM. DRY WT.	UNFREEZABLE WATER IN GM./GM. DRY WT.	UNFREEZABLE WATER IN PER CENT.
	gm.	*gm.*	*gm.*	%
Fresh leaf	7.719	7.083	0.625	8.09
1-week-old leaf	6.586	6.055	0.530	8.27
2 " " "	6.443	6.019	0.423	6.52
3 " " "	9.026	8.332	0.698	8.05
4 " " "	12.047	10.892	0.655	5.78
5 " " "	12.293	11.890	0.402	3.41
6 " " "	10.745	10.135	0.610	5.81
7 " " "	11.400	10.567	0.833	7.32
8 " " "	9.088	8.372	0.717	7.88
9 " " "	10.420	9.787	0.632	6.07
10 " " "	11.811	11.093	0.718	6.09
11 " " "	11.565	10.737	0.827	7.17
12 " " "	7.802	7.307	0.505	6.44
13 " " "	5.768	5.061	0.707	12.26

Roots appeared after the second week, and shoots after the fourth week (fig. 1, A, B). The most vigorous growth of the shoots took place during the ninth and tenth weeks. After 10 weeks of drying the central portion of the parent leaf was tough and brown, but the edge and base became even more turgid and remained green. After 12 weeks the central region was brittle dry, and after 18 weeks the whole leaf was so dry that it would crack if picked up by the petiole, and the plantlets would fall off unless the leaves were handled with great care.

In all determinations for the first 14 weeks more total water was found in the edge and base than in the central portion of the leaf on the dry weight basis. The percentage of unfreezable water was greater in the central portion than in the tissues at the edge and base during these 14 weeks, except the fifth week, at which time the tissues contained only 7.19 per cent. in the central portion and 9.32 per cent. in the edge and base (columns 4, 7, table III). This exception is not accounted for. In this series the total water decreased during the ninth and tenth weeks as it did during the eighth week in series 2. In the tissues at the edge and base of the leaf in the eleventh week there was more total water per gram of dry weight than at any other time. This parallels the determination in series 2, varying slightly in the time intervals. The total water in the central region of the leaf, on dry weight basis, did not increase after the tenth week of drying but continued to decrease until the leaf was brittle dry. The data taken in the eighteenth week were entered in table III as the central region of the leaf since there was no green or turgid tissue in the edge and base.

TABLE III

TOTAL AND UNFREEZABLE WATER IN GRAMS PER GRAM DRY WEIGHT AND UNFREEZABLE WATER IN PERCENTAGES

MATERIAL	CENTRAL PORTION OF LEAVES			EDGE AND BASE OF LEAVES			PLANTLETS		
	TOTAL WATER IN GM./GM. DRY WT.	UNFREEZABLE WATER IN GM./GM. DRY WT.	UNFREEZABLE WATER IN PER CENT.	TOTAL WATER IN GM./GM. DRY WT.	UNFREEZABLE WATER IN GM./GM. DRY WT.	UNFREEZABLE WATER IN PER CENT.	TOTAL WATER IN GM./GM. DRY WT.	UNFREEZABLE WATER IN GM./GM. DRY WT.	UNFREEZABLE WATER IN PER CENT.
	gm.	*gm.*	%	*gm.*	*gm.*	%	*gm.*	*gm.*	%
Fresh leaf	5.611	0.839	14.96	6.160	0.664	10.80	.....	.....	.....
1-week-old leaf	6.004	0.600	10.00	7.135	0.597	8.39	.....	.....	.....
2-weeks-old leaf	5.206	0.523	10.10	5.540	0.519	9.37	.....	.....	.....
3 " " "	5.205	0.542	10.41	5.727	0.336	5.39	.....	.....	.....
4 " " "	6.187	0.417	6.75	6.466	0.293	4.54	.....	.....	.....
5 " " "	6.401	0.459	7.18	7.302	0.680	9.32	5.997	0.696	11.81
6 " " "	7.405	0.546	7.37	10.257	0.333	3.27	6.411	0.741	11.56
7 " " "	8.295	0.472	5.72	11.473	0.316	2.76	6.256	0.453	7.24
8 " " "	7.733	0.384	4.97	11.278	0.423	3.76	6.670	0.402	6.02
9 " " "	6.601	0.394	5.97	9.556	0.368	3.80	6.472	0.551	8.52
10 " " "	6.748	0.412	6.11	9.179	0.345	3.75	5.962	0.529	8.87
11 " " "	6.560	0.835	9.15	13.269	0.895	6.74	6.340	0.597	9.41
12 " " "	4.000	0.399	9.98	10.145	0.728	7.19	5.982	0.343	5.73
14 " " "	0.170	0.117	68.82	1.451	0.236	18.47	3.675	0.279	7.06
18 " " "	0.130	0.063	48.82	.....	.....	.....	4.213	0.427	10.14

The percentage of unfreezable water was greater in the central portion of the leaf in each determination, with the exception of the fifth week, than in the tissues at the edge and base (columns 4, 7, table III).

The first determination on the plantlets was made after the leaves had dried for 5 weeks. The total water per gram of dry weight is less in the young plants from the fifth to tenth weeks than in the central region or the tissues along the edge and base, but after 11 weeks the plantlets had more total water than the central portion but less than was found in the tissues along the edge and base of the leaf. At 18 weeks the plantlets contained about thirty-two times as much total water as the leaf, on the dry weight basis.

The percentage of unfreezable water in the young plants is greater from the fifth to the eleventh weeks than in the parts of the parent leaf examined (columns 4, 7, 10, table III). From the twelfth to the eighteenth weeks there was a smaller percentage of unfreezable water in the plantlets than in the leaf. The greatest percentage of unfreezable water in the plantlets was found during the fifth and sixth weeks.

The data in table III are averages of four determinations on three leaves where material was available.

Measurements on cuticle thickness and distribution of stomata on leaves

Since *Bryophyllum* has the ability to retain its water to a remarkable degree, the cuticle and stomata were examined to determine their rôle in this water-retention if possible. The cuticle was examined from prepared slides, and the stomata were counted from impressions made of celloidin peels of the upper and lower surfaces of the leaves.

The cuticle of the upper epidermis was 4 μ thick and that of the lower epidermis only 2 μ. On the upper epidermis of a mature leaf there were 20 stomata per mm.^{2} and on the lower 46 per mm.^{2} On the leaf of a plantlet there were 20 stomata per mm.^{2} on the upper and 30 on the lower epidermis. The cuticle is no heavier than on most mesophytes. If the average number on the upper epidermis of a mesophyte is 120 per mm.^{2} and the average for the lower is 140 then the leaves of *Bryophyllum* have fewer stomata than mesophytes. This cuticle and epidermis of the mature leaf and the plantlet could hardly be thought of as responsible for the water-retaining properties of *Bryophyllum*.

Turrell (**22**) reports that the ratio of the internally exposed surface to the externally exposed surface of a leaf of *Bryophyllum* is only 7.8. This is the lowest ratio he reported in all of the plants he examined. This feature of the leaf structure may account in part, at least, for the ability of the leaf to hold its water against drying during an 18-week period. The internally

exposed surface, with the reduced number of stomata, may be in part responsible for the water-retaining capacity of the leaf.

Discussion

The distribution of total water in the whole plant, when the leaves and portions of the stem were examined, indicate that more total water per gram of dry weight was contained in the younger portions of the stem than in the leaves attached to those portions (column 2, table I). There was a higher percentage of unfreezable water in the second and third pairs of leaves than in the second and third internodes of the stem. As the stem became drier in the lower internodes the unfreezable water increased (column 5, table I). The bud, with the first set of leaves and the first internode, contained more total water than any of the leaves except those at the eighth node, which appeared to be water storage organs. The plants used were potted during the late autumn, and the leaves at the eighth node were smaller and thicker, never increasing in size as did the younger leaves.

In these plants in which the whole leaf and stem portions were examined, a large amount of total water was accompanied by a small amount of unfreezable water. The freezable water increased as the total water increased but the unfreezable water decreased, and conversely as the total water decreased the unfreezable water increased. In the leaves this relationship was less striking than in the stem.

When the whole leaf was examined during a period of drying, the total water and freezable water appeared to increase from the third to the eleventh week of drying (columns 2, 3, table II). This apparent increase of water must have been due to an actual decrease in the dry weight, since it is highly improbable that the leaves would take up more water from the atmosphere than they could from a plant growing in well-watered soil.

MOTHES (**14**) states that there is a greater respiration intensity in wilted leaves than in normal ones. He reported that in *Helianthus* there was a translocation of materials from the older leaves to the younger until the older leaves were exhausted. Any translocation that takes place in the detached leaves of *Bryophyllum* must be from one part of the leaf to another or to the young plantlets on the margin of the leaf. Translocation cannot take place to anything outside of the leaf or the plantlets, and the loss in dry weight must be due to respiration. The greatest loss in dry weight, as measured by the increase of total water in grams per gram dry weight, took place during or immediately following the most vigorous growth of the plantlets at the margin of the leaf. During the period of loss in dry weight the freezable water increased but the unfreezable water decreased.

As the leaf dries, in the manner described in series 2, the unfreezable water decreases until about the fifth week of drying (column 5, table II).

This decrease is not a steady decrease but an irregular one. After the fifth week the percentage of unfreezable water increases, not steadily but irregularly, until at 13 weeks it reaches 12.26 per cent., which is the highest found in the drying leaves. This final percentage is 4.17 per cent. above that of the fresh leaves examined 13 weeks before. The total water per gram of dry weight is 5.768 gm. after 13 weeks of drying as compared with 7.719 gm. in the fresh leaf (column 2, table II). This is one-fourth less total water in the 13-weeks-old leaf than in the fresh leaf on the dry weight basis.

Thus, it might at first appear that there was an increase in the percentage of unfreezable water in the process of severe drying. Almost one-half of the increase took place in the last week. The percentage of unfreezable water was 6.44 per cent. after twelve weeks of drying or 1.65 per cent. less than in the fresh leaf. It was between the determinations made the twelfth and thirteenth weeks that the most rapid drying took place, and the percentage of unfreezable water increased most during the thirteenth week. It therefore appears that the increase in percentage of unfreezable water is not responsible for the capacity of *Bryophyllum* leaves to retain water during severe drying, but rather increased merely as the result of this drying process.

The results of the measurements in series 3 indicate a shift of the total water from the central portion of the leaf to the edge and basal tissues. In the fresh leaf there were 5.611 gm. of total water in the central portion and 6.160 gm. in the edge and base per gram of dry weight (columns 2, 4, table III). At 6 weeks there were 7.405 gm. in the central portion and 10.257 gm. in the edge and base, and at 11 weeks 6.56 gm. in the central region and 13.269 gm. of total water in the edge and base per gram of dry weight. The ratios of total water in the marginal and basal tissues to the central tissues are: in fresh leaves, 1.09; after 6 weeks, 1.37; 11 weeks, 2.02; and at 14 weeks, 8.65. The ratios of grams of unfreezable water in the edge and base to the central region are: in fresh leaves, 0.72; after 6 weeks, 0.43; 11 weeks, 0.74; and 14 weeks, 0.27. Thus the ratio of unfreezable water in the edge and base of the leaf to the central portion decreases markedly as the leaf dries. Since the edge and base resist drying longer than the central region and if the unfreezable water was responsible for this resistance, then the ratios should increase. This was found not to be the case.

The movement of the water from the central region to the edge and basal tissues will maintain the young plants at the margin of the leaves. The increased unfreezable water in the central tissues of the leaf seems to be the result of the shift of the total water to the marginal and basal tissues rather than a means for the shift. The shift may be caused by greater osmotic values of the tissues at the margin. FREELAND (**4**) found in some of the leaves of *Bryophyllum* that were producing new plants at the margin, a greater osmotic value at the margin than in the central region.

In the plantlets the total water in grams per gram of dry weight was observed to be less than that of the leaf from the fifth to the eleventh week (column 8, table III). However, at the end of 18 weeks there were 4.213 gm. of total water in the plantlets and only 0.130 gm. in the parent leaf on the dry weight basis. The plantlets remained turgid and green while the parent leaf was brittle dry. The percentage of unfreezable water in the plantlets decreased from 11.81 per cent. in the very young plants to 5.73 per cent. after drying for 7 weeks, then increased to 10.14 per cent. after drying for 13 weeks (column 10, table III). If the unfreezable water were responsible for the ability of these plantlets to retain their water it should increase, if not from the first determination then certainly after 13 weeks of drying. This was not the case. In the plantlets there is less relation between the severe drying and the unfreezable water than in the drying leaf.

It appears that the unfreezable water in *Bryophyllum* leaves increases with the severe drying of the leaves and cannot be responsible for the water-retaining capacity of the leaf and plantlets. The unfreezable water shows less correlation to the drying in the plantlets than in the parent leaf.

Summary

1. The term freezable water is used to designate that state of water referred to by some authors as "free" water, and unfreezable water as that referred to as "bound" water.

2. Since measurements of unfreezable water in the tissues of plants had been frequently employed in attempts to interpret the changes occurring in cold hardening, and very little had been done on drought resistance in relation to the unfreezable water content of tissues it seemed desirable to determine the total, freezable, and unfreezable water in *Bryophyllum* leaves during severe drying.

3. In the whole plant in general there was a correlation between the age of the tissue examined and the percentage of unfreezable water it contained, that is, the older the tissues the greater the percentage of unfreezable water. The bud, however, contained less unfreezable water than did tissues of intermediate age.

4. As the leaf dries out the percentage of unfreezable water increases as a result of the loss of freezable water by evaporation. The increase in the unfreezable water does not seem to the writer to be responsible for the water-retaining ability of the leaf but rather a result of the drying.

5. In the plantlets, where there is the greatest ability to retain the water against atmospheric drying, there is less correlation between the unfreezable water and the drying than in the drying of the parent leaf. The youngest plants on the margin of the leaves have the greatest percentage of unfreezable water.

6. The reduced internally exposed surface and the reduced number of stomata may be responsible for the ability of *Bryophyllum* leaves to retain their water.

The writer wishes to thank DR. C. A. SHULL for suggestions and criticisms during this investigation. Thanks are also due the Otho S. A. Sprague Memorial Institute of the University of Chicago for the use of their equipment, and the members of its staff for assistance in assembling it.

UNIVERSITY OF CHICAGO

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ASSIMILATION OF AMMONIUM AND NITRATE BY PINEAPPLE PLANTS GROWN IN NUTRIENT SOLUTIONS AND ITS EFFECTS ON NITROGENOUS AND CARBOHYDRATE CONSTITUENTS[1, 2]

C. P. Sideris, B. H. Krauss, and H. Y. Young[3]

(WITH TWENTY-TWO FIGURES)

Introduction

The nutrition of pineapple plants, *Ananas comosus* Merr., with either ammonium or nitrate salts is exceedingly interesting from the point of view of plant growth and performance. Because the complexity of factors involved in a problem of this type is great and a satisfactory solution is difficult without recourse to methods offering greater control of the external nitrogen supply than can be obtained by growing plants in soil, the studies presented in the following pages were carried out with plants grown under greenhouse conditions in complete nutrient solutions containing either ammonium or nitrate nitrogen. In a subsequent publication similar data will be presented on plants grown under various other conditions and fertilized with either ammonium sulphate or sodium nitrate.

Stewart, Thomas, and Horner (**14**) studied the ammonium and nitrate nutrition of pineapple plants with results which may be stated briefly as follows: "The best growth was made by plants growing in the normal nitrate nitrogen cultures; those in one-fourth normal cultures made a very poor growth. Under the conditions of this experiment ammonia nitrogen was not readily utilized from the full strength normal nutrient solution. The plants in the ammonia cultures diluted to one-fourth made a better development than those in the full strength solutions. This would appear to suggest that under field conditions the pineapple plant probably uses nitrogen in both the nitrate and ammoniacal forms." Although the studies of these investigators indicate the direct assimilation of ammonium without previous conversion into nitrate, they have left untouched the phases concerned with the movement and assimilation of ammonium and nitrate nitrogen in the various organs and tissues of the plant.

In 1935 the senior author reported (**12**) various studies concerned with the relative degree of absorption and movement of nitrate nitrogen and its assimilation by the various sections of pineapple plants. The studies men-

[1] Published with the approval of the Director as technical paper no. 105 of the Pineapple Experiment Station, University of Hawaii.

[2] The second of a series on ammonium and nitrate nutrition.

[3] Presented in part by the senior author in the meetings of the American Society of Plant Physiologists at St. Paul, Minnesota, in June, 1935.

tioned indicated that the assimilation of nitrate in the roots, stem, and white basal leaf tissues was extremely slow. In the green tissues of the leaves the rate of nitrate assimilation was very rapid.

Some of the more outstanding contributions on ammonium and nitrate nutrition have been presented in the first paper of this series (**13**). For a very comprehensive and thorough review of the literature of nitrogen nutrition the reader is referred to a recent publication by NIGHTINGALE (**8**).

Methods of experimentation

CULTURAL METHODS

Clon planting material of uniform weight and appearance was employed. The plants were rooted and grown in Mason jars filled with 1800 cc. of nutrient solution containing either ammonium or nitrate as nitrogen sources. The chemical composition of the solution containing ammonium nitrogen is shown in table I.

TABLE I

COMPOSITION OF AMMONIUM NUTRIENT SOLUTION

SALTS USED	PARTS PER MILLION							
	N	P	K	Ca	Mg	Cl	S	Fe
Ammonium sulphate	28						32	
Potassium phosphate		15	20					
Potassium sulphate			40				32	
Calcium chloride				40		70		
Magnesium sulphate					24		32	
Iron sulphate							3	5
Microelements: B, Zn, Mn, Al, Si, Cu, Ti, Br, I, 0.5 to 1.0 p.p.m. each								
Total	28	15	60	40	24	70	99	5

The solution was initially adjusted to pH 5.4 and was maintained at pH 5.0 $\pm$ 0.6 by small quantities of 0.1/N NaOH, added at intervals as required.

The composition of the solution containing nitrate nitrogen is shown in table II.

The solution was originally adjusted to pH 4.8 and was maintained at pH 4.8 $\pm$ 0.6 with the addition of calculated amounts of 0.1/N H_2SO_4 as required.

The concentrations of the various ions of both nutrient solutions, when compared with those of other well known complete solutions, were very low. The reasons for adopting these low concentrations were (1) the relatively low rate of growth of the pineapple plant; and (2) the comparatively high temperature conditions of the greenhouse during the summer months. Both conditions favor abnormally great accumulation of salts in plant tissues.

TABLE II

COMPOSITION OF NITRATE NUTRIENT SOLUTION

SALTS USED	PARTS PER MILLION							
	N	P	K	Ca	Mg	Cl	S	Fe
Calcium nitrate	28			40				
Potassium phosphate		15	20					
Potassium sulphate			40				32	
Magnesium sulphate					24		32	
Iron sulphate							3	5
Microelements: B, Zn, Mn, Al, Si, Cu, Ti, Br, I, 0.5 to 1.0 p.p.m. each								
Total	28	15	60	40	24	0	67	5

The nutrient solutions were changed weekly, at which time the volume of absorbed and evaporated water and the weight of the plants were determined. At the time of harvest the plants were about four and a half months old.

METHODS OF SAMPLING

For the preparation of the plant tissues for chemical analysis the leaves were detached from the stem and divided, on the basis of maturity, into four groups. These groups were further subdivided into sections of different age and physiological function as indicated.

LEAF AND STEM GROUPING AND SECTIONING.—Pineapple plants (fig. 1) are propagated under field conditions from three asexually produced vegetative organs known respectively as suckers, slips, and crowns, each of which re-

A B

FIG. 1. Pineapple plants (A) before, and (B) after development.

quires a different period from planting to fruiting on account of differences in initial weight and stage of ontogenetic development. The initial number, length, and weight of the leaves and the length, diameter, and weight of the stem of these various types of propagating material vary. The leaves which are produced at successive intervals after planting increase in length, width, and weight until the fruiting stage is reached, as shown in figure 1-B. All leaves formed during the latter stage are comparatively small and are borne on the peduncle. Pineapple plants bear leaves simultaneously with stages of development ranging from embryonic to senescent. Similar differences in developmental stage may be found between the tissues of the basal and terminal sections of the same leaves. The basal sections, on account of their association with meristematic tissues, are from the point of view of anatomical and functional development embryonic, while the terminal sections are senescent.

With these facts under consideration we divide the entire leaf system of the plant, as well as possible, into chronologically homogeneous groups, as shown in figure 2, where 25 per cent. of the representative leaves of each

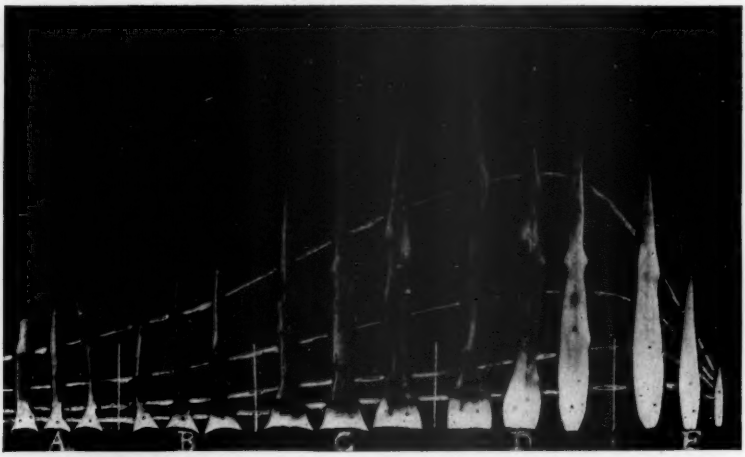

FIG. 2. Different leaf groups, and sections, as indicated with the white lines.

group are shown. Each group was then subdivided into morphologically and physiologically homogeneous sections as shown in figures 2 and 6. The stem was also divided into sections, each one of which corresponded to and was delineated by the region of attachment of the respective leaf groups as shown in figure 4.

The characteristic morphological features of leaves associated with the

chronological development of the different groups of six-months-old plants may be stated as follows:

LEAF GROUPS.—

A *Old leaves of the planting material.*—These are the leaves which, after planting, produce no new growth, as attested by the absence of groups of lateral spines at distances slightly above the base (figs. 1-a, 2, 6).

B *Leaves of the planting material with new growth.*—They represent

FIG. 3. A leaf of the B group showing spines and constrictions.

leaves already present on the planting material and which after planting produce new growth as attested by the lateral spines and the point of constriction of the leaf (figs. 1, 2, 3, 6).

C *Mature leaves produced after planting.*—The most mature of the new leaves. They have broad bases containing little or no succulent-brittle non-chlorophyllous tissue and have lateral points at the base decidedly expanded.[4] Their number varies from 6 to 15 according to plant age (figs. 1, 2, 5, 6).

[4] "Expanded" indicates stretched condition.

D *Active or longest leaves.*—The longest leaves on the plant number from 6 to 12, depending on plant-size and age. In non-fruit-bearing plants, the leaf-base consists of succulent-brittle non-chlorophyllous tissues, slightly broader than the green blade proper, and with non-expanded lateral points; while in fruit-bearing plants it lacks succulent-brittle non-chlorophyllous tissues, is tough and with lateral points prominently expanded (figs. 1-a, 2, 5, 6).

FIG. 4. External (left) and internal (right) view of a stem.

E *Immature or shoulder leaves.*—These are borne on the shoulder of the stem (transition zone from stem proper to peduncle) and may number from 6 to 12, depending on plant age and size. In non-fruit-bearing plants the base of the leaves is narrower than the green blade proper and contains great amounts of succulent-brittle non-chlorophyllous tissues. In fruit-bearing plants these leaves occupy the region between the suckers and the base of the peduncle. Then their bases become broader than the green blade proper, and lack succulent-brittle non-chlorophyllous tissues (figs. 1, 2, 6).

F_1 *Youngest or apical leaves.*—The youngest leaves on the plant in stages preceding peduncle formation. They may number from 9 to 18, depending on the size and age of the plant. The width of their base is either the same or slightly narrower than that of the green blade proper (figs. 1, 2, 6).

The nomenclature adopted for the different leaf sections is based on certain morphological characters and on chlorophyll distribution. They are shown in figure 5 and are as follows:

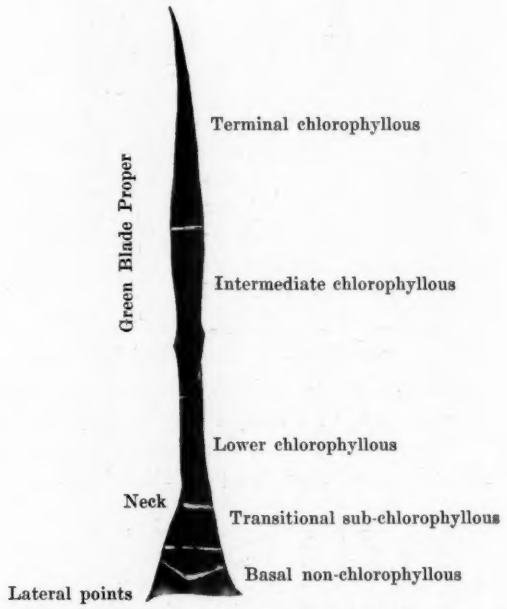

FIG. 5. Nomenclature of the different sections and other parts of a leaf.

LEAF SECTIONS.—

1 *Basal-non-chlorophyllous.*—The white basal tissues of leaves. (It varies considerably in area and weight in the respective groups of leaves according to their stage of development.)

2 *Transitional sub-chlorophyllous.*—The transition region between the chlorophyll-free base and the chlorophyllous tissues of the green blade proper, better delimited in mature leaves by the neck, and in immature ones by the lower limit of the chlorophyllous tissues.

3 *Lower chlorophyllous.*—Approximately the lower third of the green blade proper.

4. *Intermediate chlorophyllous.*—Approximately the middle third of the green blade proper.

5 *Terminal chlorophyllous.*—Approximately the terminal third of the green blade proper.

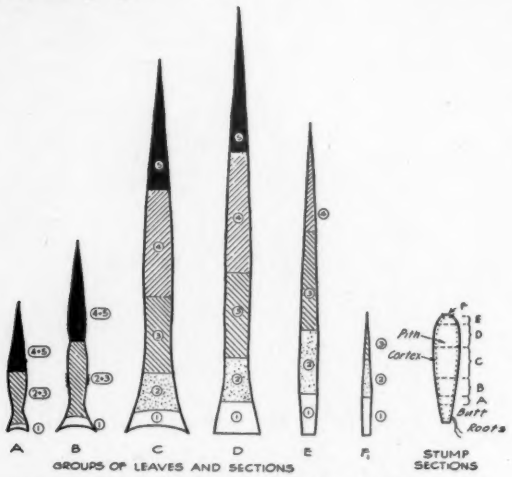

FIG. 6. Diagrammatic representation of the leaves, etc.

The relative amounts of the tissues making up each plant section that was employed for an analytical sample may be obtained by referring to figures 6 and 7 where the different sections are well defined and the magnitude of each section is represented on a percentage basis.

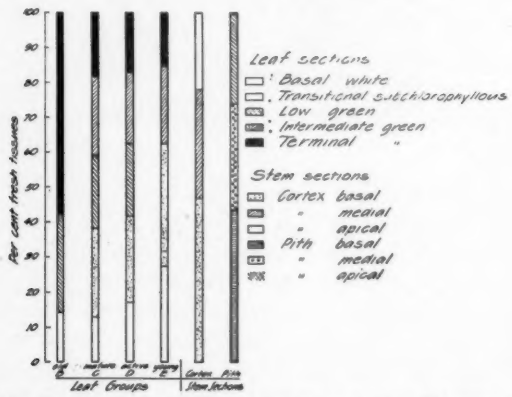

FIG. 7. Relative percentage distribution of the weights of the various leaf and stem sections which were each employed as separate analytical samples.

Analytical methods

For each sample between 25 and 50 grams of fresh tissues, obtained from each section of the plant as described, were shredded and placed in flasks containing about 100 ml. of boiling water, and kept boiling for a period of 20 to 40 seconds, to inactivate any enzymes present. The flasks were then allowed to cool and toluene was added. Shortly afterwards, or at most in the course of a month, the various samples were ground in a brass mortar with quartz sand and analysed for different fractions of inorganic, soluble organic, and protein nitrogen, and for total and reducing sugars. The methods employed for the analysis of different nitrogenous fractions and sugars have been presented and discussed in the first paper of this series (**13**). Total sugars were determined after hydrolysis with invertase.

Chlorophyll was extracted from fresh tissues and was separated from the carotenoid pigment according to SCHERTZ's method (**9**). It was then determined colorimetrically using GUTHRIE's (**2**) standard.

The water content of the various sections was determined after drying fresh tissues of known weights in a drier at about 100° C.

Results

Rate and amount of growth, water absorption, moisture and chlorophyll content

As plotted against time the growth curve of pineapple plants (fig. 8)

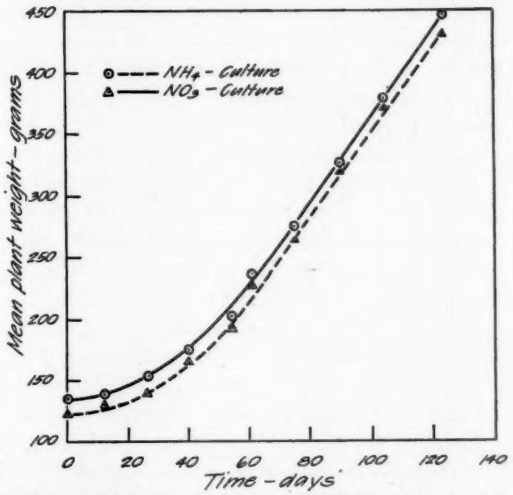

FIG. 8. Relative growth in grams of plants at different stages of development with either ammonium or nitrate nutrition.

five months old is J-shaped, because it represents only about one-half of the growth cycle of the plant. For plants having completed their growth cycle, a sigmoid curve, resembling that of autocatalysis, is obtained. Such a curve was obtained by STEWART, THOMAS, AND HORNER (**14**) and by HORNER (**4**) for plants grown under field conditions.

TABLE III

MEAN WEIGHT OF PLANTS (M) AT DIFFERENT STAGES OF DEVELOPMENT GROWN IN SOLUTIONS WITH EITHER AMMONIUM OR NITRATE NUTRITION, TOGETHER WITH VALUES OF STANDARD DEVIATION (σ), COEFFICIENT OF VARIABILITY (C), AND PROBABLE ERROR (E) OF THE MEAN

DATE	AMMONIUM N.				NITRATE N.			
	M	σ	C	E	M	σ	C	E
	gm.		%		*gm.*		%	
Dec. 26	124	19.6	15.8	± 3.8	134	10.4	8.0	± 2.0
Jan. 7	130	20.2	15.5	± 3.8	141	14.7	10.5	± 2.8
Jan. 21	140	22.0	15.8	± 4.1	153	16.0	10.4	± 3.0
Feb. 4	164	25.0	15.2	± 4.7	175	17.1	9.8	± 3.2
Feb. 18	193	30.0	15.5	± 5.7	203	22.0	10.7	± 4.1
Feb. 25	227	37.4	16.5	± 7.3	237	33.0	13.9	± 6.4
Mar. 11	266	40.0	15.0	± 7.8	276	34.1	12.3	± 6.6
Mar. 28	320	47.0	14.9	± 9.2	325	38.7	11.9	± 7.6
Apr. 11	368	51.0	13.9	± 10.0	381	48.0	12.6	± 9.4
Apr. 30	430	56.7	13.2	± 11.0	446	52.4	11.8	± 10.2

In table III the mean weight values of the ammonium and nitrate series of plants together with their statistical treatment are presented. The variability (C) in the plants of both series ranged, throughout their entire growth, from 8 to 16 per cent. The slightly greater variability in the ammonium- than in the nitrate-supplied plants was caused in certain cases by an accidental infection by pythiaceous organisms which produced a greater amount of root rot in the ammonium than in the nitrate series. Differences between the two series of plants in rate and amount of growth are insignificant, indicating that either ammonium or nitrate salts promoted growth at practically the same rate, provided other conditions were favorable.

The volume of water absorbed from the nutrient solution for every gram of fresh tissue produced (the *transpiration intensity* of the plant) was greater in the ammonium than in the nitrate series as shown in figure 9. The values obtained agree fairly well with those obtained by KRAUSS (**5**). The difference in transpired water between the two lots of plants is correlated with the greater tissue succulence of plants of the ammonium series. The moisture content of the various sections is reported in figure 10. The values for the ammonium series are in general higher than those for the nitrate-supplied plants, indicating a greater degree of succulence for plants of the ammonium series.

The distribution of chlorophyll in the various sections of leaves varies considerably as shown in figure 11. The amounts for corresponding tissues are greater in 11-A than in 11-B indicating that ammonium more than nitrate nutrition favored the formation of chlorophyll. The factors responsible for greater amounts of chlorophyll in the plants of the ammonium than in those of the nitrate series probably are the greater amounts of absorbed

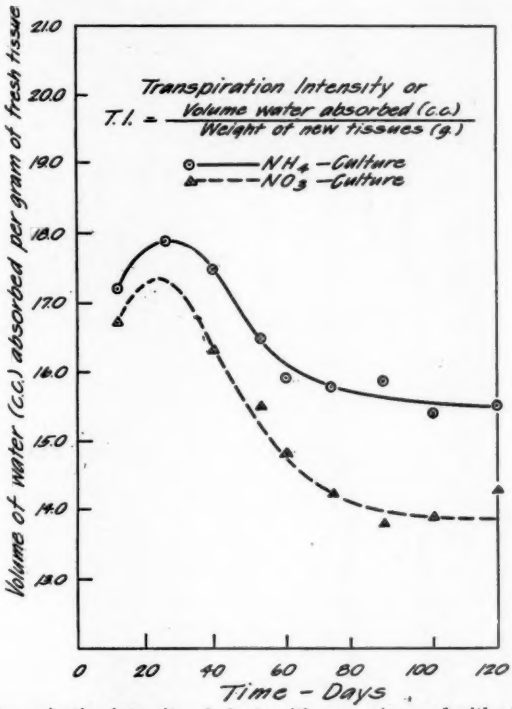

FIG. 9. Transpiration intensity of plants with ammonium and with nitrate nutrition.

nitrogen and the higher acidity in the nutrient solution and on the surface of the roots, caused at least in part by unequal absorption of ammonium and sulphate ions. Under such conditions iron and phosphorus as well as certain other elements would remain in solution and therefore be more available. The residual effect in the case of nitrate nutrition is that of decreased acidity owing to a more rapid absorption of the nitrate than the calcium ions.

With higher amounts of chlorophyll in the leaves synthesis of greater quantities of sugars might be anticipated. However, the quantities of sugars in the tissues of the ammonium series of plants with higher amounts of

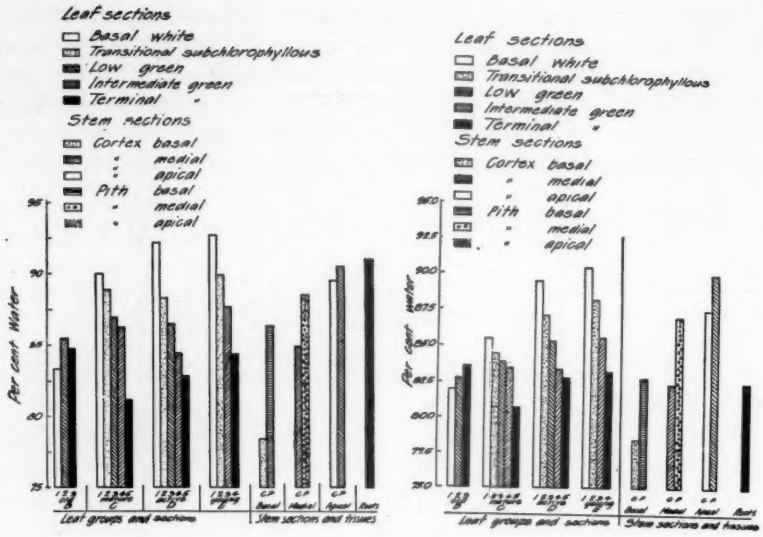

FIG. 10. Percentage water content of various sections of pineapple plants with ammonium nutrition (A), and with nitrate nutrition (B).

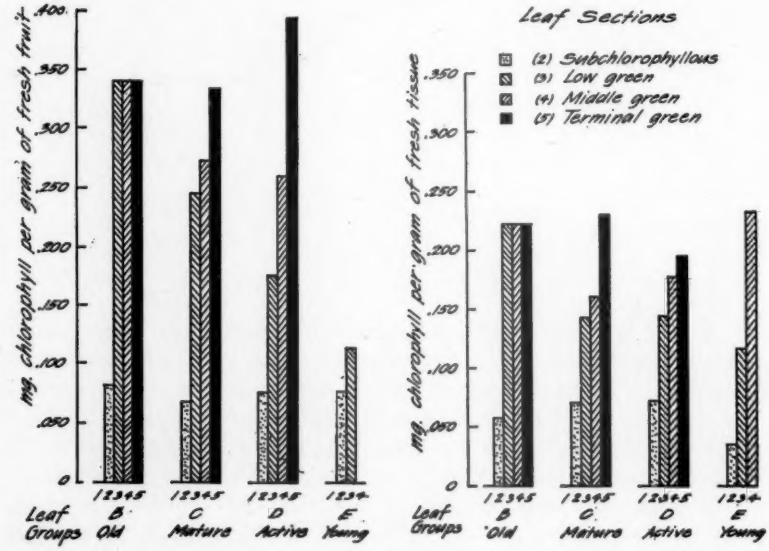

FIG. 11. Chlorophyll content of various leaf sections with ammonium nutrition (A), and nitrate nutrition (B).

chlorophyll were not any greater than those of the nitrate series. This may well have been caused in part by the great utilization of sugars, or their derivatives, in organic nitrogen synthesis, which, as will be subsequently shown, took place at a relatively higher rate in the leaves of the ammonium group.

Relative absorption of ammonium and nitrate nitrogen

Our discussion of the work of other investigators (**13**) has indicated that ammonium is a better source of nitrogen under field conditions than nitrate. In culture solutions Stewart, Thomas, and Horner (**14**) have pointed out that nitrate in high, and ammonium in low concentrations were satisfactory sources. The analytical data of these investigators (p. 234, table 5), however, show that the quantities of nitrogen in the tissues of the plants grown either in high or low ammonium or nitrate cultures were consistently greater in the plants with ammonium nutrition, in spite of the adverse statements of these authors that ammonium in the "normal strength" culture was not readily utilized.

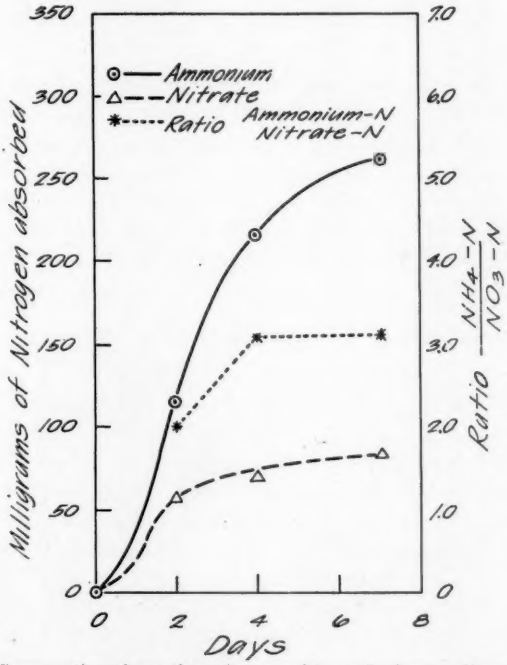

Fig. 12. Comparative absorption of ammonium and nitrate nitrogen by pineapple roots on a per plant basis.

Accordingly, an experiment on direct nitrogen absorption was established to measure the quantities of ammonium or nitrate nitrogen absorbed as such from the nutrient solution. Plants with highly uniform root and leaf systems were selected and placed in containers with either ammonium or nitrate nitrogen in equal amounts. Light and temperature conditions and the initial pH 5.0 ± 0.4 were as nearly alike as possible in both cases. At intervals of 2, 4, and 7 days the quantities of nitrogen removed were determined by analysis of the residual culture solutions. The results obtained are reported in table IV, and figure 12.

TABLE IV

MEAN VALUES OF ABSORBED NITROGEN PER PLANT FROM SOLUTION CULTURES WITH EITHER AMMONIUM OR NITRATE NITROGEN

DAYS	ABSORBED NITROGEN AS		RATIO NH_4–N / NO_3–N
	AMMONIUM	NITRATE	
	mg.	*mg.*	
2	116	57	2.0
4	215	69	3.1
7	260	83	3.1

The values of absorption of either ammonium or nitrate nitrogen, as stated in table IV, do not apply to the entire growth cycle of the plant but only to that part between the second and third months after planting. That this rate of absorption is maintained throughout the entire growth cycle is very doubtful. Analytical data, however, have shown in certain of the cases already mentioned and in those reported below that the plants absorbed nitrogen more rapidly from the ammonium than from the nitrate cultures.

DISTRIBUTION OF THE ABSORBED NITROGEN IN THE PLANT

The terms inorganic and unassimilated nitrogen are synonymous. When inorganic nitrogen either as ammonium or nitrate enters the roots, it may be converted immediately into organic nitrogen or it may be translocated through the water conducting tissues to the stem and then to the leaves. Organic nitrogen is segregated for convenience on the basis of whether it is soluble or insoluble in water. Soluble organic nitrogen comprises such fractions as amide (glutamine and asparagine), mono-amino (mostly nitrogen of alpha-amino acids), basic (mostly nitrogen from diamino acids) and rest nitrogen (undetermined compounds). Insoluble organic nitrogen is composed almost exclusively of protein. In the analyses which follow, proteins have been hydrolysed with sulphuric acid and the resulting fractions determined separately.

In tables V and VI are presented all the data on the analyses of the various tissues of both series of plants. Tables VII and VIII contain practically the same data as V and VI except that the values of the different nitrogenous fractions are reported on a percentage basis, which facilitates comparison. Figures 13-A to 18-A illustrate graphically the quantities of the different

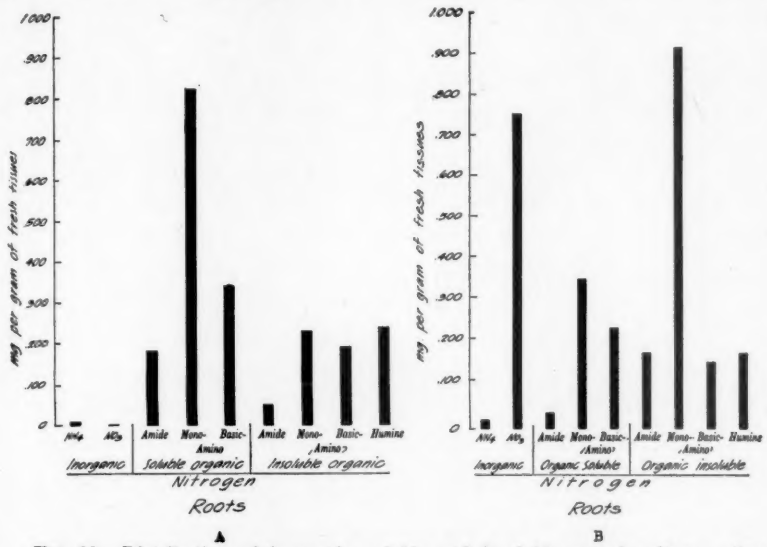

FIG. 13. Distribution of inorganic, soluble and insoluble organic nitrogen fractions in the roots of plants with ammonium nutrition (A), and nitrate nutrition (B).

fractions of nitrogen in the various sections of the plants supplied with ammonium nitrogen, and figures 13-B to 18-B illustrate the nitrogen composition of plants supplied with nitrate nitrogen.

ROOTS.—The outstanding results in the chemical composition of the root tissues of the ammonium and nitrate cultures (fig. 13) are: (a), very small amounts of ammonium in the roots of plants supplied with this nutrient, indicating a very high rate of assimilation of ammonium; (b), very great quantities of nitrate in the roots of plants with nitrate nutrition, indirectly indicating that it is absorbed fully but assimilated at a relatively slow rate; (c), large amounts of soluble organic nitrogen in the roots of the ammonium-supplied plants and comparatively small quantities in those of the nitrate cultures, results clearly in harmony with the relative amounts of (absorbed) ammonium and nitrates; and (d), very great quantities of insoluble organic nitrogen in the plants receiving nitrate nutrition and the low quantities in those of the ammonium cultures suggesting in the latter

TABLE V

DISTRIBUTION OF INORGANIC, SOLUBLE ORGANIC AND INSOLUBLE ORGANIC NITROGEN FRACTIONS IN THE DIFFERENT SECTIONS OF THE LEAVES, STEM AND ROOTS OF *Ananas comosus* (L.) MERR. GROWN IN AMMONIUM SOLUTION CULTURES

	Plant tissues	Milligram of nitrogen per gram of fresh tissues										Total organic
		Inorganic		Soluble organic				Insoluble organic				
		Ammonium	Nitrate	Glutamine	Asparagine	Monoamino	Basic	Amide	Monoamino	Basic	Humine	
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Leaves:*												
Old	B–1 (base)	0.003	0	0.003	0.132	0.240	0.285	0.128	0.243	0.112	0.189	1.332
	2	0.006	0	0.002	0.094	0.200	0.253	0.067	0.231	0.071	0.115	1.032
	3 (tip)	0.008	0	0.007	0.144	0.470	0.534	0.074	0.461	0.141	0.211	2.041
Mature	C–1 (base)	0.002	0	0.038	0.008	0.286	0.202	0.114	0.290	0.040	0.054	1.032
	2	0.002	0	0.027	.....	0.135	0.142	0.107	0.472	0.101	0.088	1.082
	3	0.001	0	0.058	0.060	0.260	0.188	0.175	1.060	0.195	0.168	2.164
	4	0.002	0	0.013	0.030	0.382	0.303	0.278	1.448	0.290	0.202	3.026
	5 (tip)	0.002	0	0.037	0.064	0.378	0.303	0.320	1.630	0.317	0.222	3.231
Active	D–1 (base)	0.002	0	0.030	0.112	0.490	0.371	0.099	0.343	0.074	0.054	1.573
	2	0.001	0	0.026	0.008	0.164	0.148	0.129	0.626	0.108	0.088	1.297
	3	0.002	0	0.028	0.020	0.233	0.236	0.266	1.185	0.208	0.135	2.311
	4	0.002	0	0.016	0.060	0.370	0.384	0.288	1.360	0.283	0.189	2.950
	5 (tip)	0.001	0	0.022	0.046	0.500	0.445	0.335	1.930	0.310	0.243	3.830
Young	E–1 (base)	.....	0	0.011	0.236	0.566	0.512	0.093	0.410	0.115	0.086	2.029
	2	0.001	0	0.018	0.204	0.328	0.258	0.070	0.301	0.099	0.099	1.377
	3	0.002	0	0.014	0.186	0.572	0.466	0.147	0.505	0.168	0.199	2.255
	4 (tip)	0.008	0	0.008	0.180	0.792	0.552	0.096	0.589	0.218	0.294	2.729
Stem:*												
Base	B–Cortex	0.009	0	0.009	0.144	0.206	0.224	0.051	0.231	0.099	0.179	1.143
	Pith	0.005	0	0.010	0.204	0.193	0.249	0.042	0.083	0.048	0.074	0.903
Middle	C–Cortex	0.008	0	0.011	0.236	0.480	0.441	0.042	0.269	0.102	0.173	1.744
	Pith	0.013	0	0.007	0.180	0.497	0.459	0.045	0.115	0.048	0.077	1.428
Tip	D–Cortex	0.009	0	0.071	0.172	0.704	0.378	0.183	0.572	0.128	0.067	2.275
	Pith	0.005	0	0.056	0.172	0.656	0.310	0.152	0.408	0.101	0.034	1.889
Roots		0.025	0	0.009	0.338	0.653	0.340	0.051	0.230	0.090	0.138	1.849

* For a more detailed description of plant parts see text.

TABLE VI

DISTRIBUTION OF INORGANIC, SOLUBLE ORGANIC AND INSOLUBLE ORGANIC NITROGEN FRACTIONS IN THE DIFFERENT SECTIONS OF THE LEAVES, STEM

Roots	0.025	0	0.009								

* For a more detailed description of plant parts see text.

TABLE VI

DISTRIBUTION OF INORGANIC, SOLUBLE ORGANIC AND INSOLUBLE ORGANIC NITROGEN FRACTIONS IN THE DIFFERENT SECTIONS OF THE LEAVES, STEM AND ROOTS OF *Ananas comosus* (L.) MERR. GROWN IN NITRATE SOLUTION CULTURES

PLANT TISSUES	MILLIGRAM OF NITROGEN PER GRAM OF FRESH TISSUES										TOTAL ORGANIC
	INORGANIC		SOLUBLE ORGANIC				INSOLUBLE ORGANIC				
	AMMONIUM	NITRATE	GLUTAMINE	ASPARAGINE	MONOAMINO	BASIC	AMIDE	MONOAMINO	BASIC	HUMINE	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Leaves:*											
Old — B–1 (base)	0.005	0.024	0.053	0.106	0.036	0.095	0.068	0.557	0.050	0.150	1.115
2	0.011	0.006	0.053	0.114	0.013	0.074	0.059	0.700	0.040	0.072	1.125
3 (tip)	0.007	0.000	0.049	0.106	0.023	0.092	0.099	0.714	0.167	0.120	1.370
Mature — C–1 (base)	0.007	0.212	0.049	0.106	0.046	0.167	0.065	0.462	0.059	0.086	1.040
2	0.006	0.056	0.058	0.116	0.031	0.116	0.068	0.492	0.044	0.086	1.011
3	0.007	0.004	0.058	0.094	0.031	0.112	0.086	0.700	0.194	0.136	1.411
4	0.005	0.000	0.058	0.100	0.011	0.115	0.119	0.980	0.307	0.153	1.843
5 (tip)	0.006	0.000	0.058	0.132	0.012	0.157	0.136	1.060	0.375	0.200	2.122
Active — D–1 (base)	0.022	0.179	0.058	0.226	0.026	0.133	0.063	0.307	0.043	0.067	0.923
2	0.007	0.144	0.053	0.120	0.026	0.121	0.100	0.590	0.067	0.086	1.163
3	0.007	0.008	0.056	0.136	0.031	0.157	0.125	0.920	0.231	0.125	1.781
4	0.008	0.000	0.053	0.130	0.009	0.148	0.183	1.160	0.260	0.176	2.121
5 (tip)	0.008	0.000	0.008	0.042	0.103	0.157	0.275	1.264	0.345	0.187	2.381
Young — E–1 (base)	0.006	0.063	0.014	0.092	0.250	0.243	.....	.....	.....	.....	.....
2	0.009	0.047	0.011	0.056	0.149	0.138	.....	.....	.....	.....	.....
3	0.009	0.006	0.006	0.040	0.104	0.108	.....	.....	.....	.....	.....
4 (tip)	0.010	0.000	0.006	0.052	0.101	0.133	0.167	1.220	0.380	0.170	2.229
Stem:*											
Base — B–Cortex	0.007	0.242	0.017	0.040	0.184	0.152	0.084	0.435	0.113	0.106	1.131
Pith	0.007	0.234	0.009	0.022	0.285	0.194	0.075	0.352	0.056	0.052	1.045
Middle — C–Cortex	0.018	0.280†	0.017	0.084	0.466	0.296	0.097	0.512	0.115	0.075	1.662
Pith	0.016	0.375	0.018	0.106	0.613	0.436	0.087	0.474	0.100	0.061	1.895
Tip — D–Cortex	0.008	0.312	0.028	0.134	0.439	0.355	0.089	0.486	0.134	0.046	1.711
Pith	0.037	0.469	0.027	0.258	0.564	0.426	0.125	0.640	0.167	0.064	2.271
Roots	0.022	0.750	0.013	0.052	0.319	0.227	0.167	0.916	0.141	0.163	1.998

* For a more detailed description of plant parts see text.
† Obtained with the xylenol method.

TABLE VII

MILLIGRAMS OF INORGANIC, SOLUBLE ORGANIC, INSOLUBLE ORGANIC AND TOTAL NITROGEN PER GRAM OF FRESH WEIGHT IN THE DIFFERENT SECTIONS OF THE PLANT, TOGETHER WITH PERCENTAGE VALUES OF THE SAME AND OF THEIR FRACTIONS AS FOUND IN PINEAPPLE PLANTS GROWN IN SOLUTION CULTURES CONTAINING AMMONIUM NITROGEN

	Plant tissues	Inorganic nitrogen				Soluble organic nitrogen						Insoluble organic nitrogen						Total N
		% of inorganic		Total	% of total	% of soluble organic				Total	% of total	% of insoluble				Total	% of total	
		Ammonium	Nitrate			Glutamine	Asparagine	Monoamino	Basic			Amide	Monoamino	Basic	Humine			
	Leaves*	%	%	*mg.*	%	%	%	%	%	*mg.*	%	%	%	%	%	*mg.*	%	*mg.*
Old	B–1 (base)	100	0	0.003	0.2	0.4	10.0	46.4	43.2	0.660	49.5	19.05	36.15	16.65	28.15	0.672	50.3	1.335
	2	100	0	0.006	0.6	0.3	8.5	45.0	46.2	0.548	52.8	13.80	47.80	14.65	23.75	0.484	46.6	1.038
	3 (tip)	100	0	0.008	0.3	0.5	6.3	47.0	46.2	1.154	56.4	8.30	52.00	15.90	23.80	0.887	43.3	2.049
Mature	C–1 (base)	100	0	0.002	0.2	7.1	0.8	54.3	37.8	0.534	51.6	22.90	58.30	8.00	10.80	0.498	48.2	1.034
	2	100	0	0.002	0.2	8.6	3.2	43.0	45.2	0.314	28.8	13.95	61.45	13.15	11.45	0.768	71.0	1.084
	3	100	0	0.001	0.1	8.9	4.6	51.2	33.3	0.566	26.1	11.00	66.30	12.20	10.50	1.598	73.8	2.165
	4	100	0	0.002	0.1	2.1	2.4	46.7	48.8	0.708	23.4	12.50	65.40	13.00	9.10	2.218	76.5	3.028
	5 (tip)	100	0	0.002	0.1	5.0	4.3	49.9	40.8	0.742	22.9	12.90	65.50	12.70	8.90	2.489	77.0	3.233
Active	D–1 (base)	100	0	0.002	0.1	3.0	5.5	54.5	37.0	1.003	63.7	17.40	60.16	12.97	9.47	0.570	36.2	1.575
	2	100	0	0.001	0.1	7.5	1.1	48.6	42.8	0.346	26.6	13.50	65.90	11.40	9.20	0.951	73.3	1.298
	3	100	0	0.002	0.2	5.4	1.9	47.0	45.7	0.604	25.4	14.80	66.10	11.60	7.50	1.794	74.6	2.313
	4	100	0	0.002	0.1	1.9	3.6	48.2	46.3	0.830	26.9	13.55	64.20	13.35	8.90	2.120	73.1	2.952
	5 (tip)	100	0	0.001	0.0	2.2	2.2	51.6	44.0	1.012	26.4	11.90	68.50	11.00	8.60	2.818	73.6	3.831
Young	E–1 (base)	100	0	.....	.....	0.9	8.9	51.6	38.6	1.325	65.3	13.20	58.30	16.30	12.20	0.704	34.7	2.029
	2	100	0	0.001	0.1	2.2	12.6	53.2	32.0	0.808	58.6	12.30	53.00	17.35	17.35	0.569	41.3	1.378
	3	100	0	0.002	0.1	1.1	7.5	53.8	37.6	1.238	54.8	14.40	49.75	16.30	19.55	1.017	45.1	2.257
	4 (tip)	100	0	0.008	0.3	0.6	5.9	57.5	36.0	1.532	56.0	8.10	49.20	18.20	24.50	1.197	43.7	2.737
	Stem*																	
Base	B–Cortex	100	0	0.009	1.2	1.4	12.4	47.7	38.5	0.583	50.3	9.10	41.20	17.70	32.00	0.560	48.5	1.152
	Pith	100	0	0.005	0.5	1.4	15.6	45.0	38.0	0.656	72.3	17.00	33.60	19.40	30.00	0.247	27.2	0.908
Middle	C–Cortex	100	0	0.008	0.5	0.9	10.1	51.2	37.8	1.168	66.3	7.17	45.90	17.40	29.50	0.586	33.2	1.762
	Pith	100	0	0.013	0.9	0.5	7.9	51.4	40.2	1.143	79.3	15.60	40.40	17.80	27.00	0.285	19.8	1.441
Tip	D–Cortex	100	0	0.009	0.4	5.4	6.5	59.6	28.5	1.325	58.0	18.30	60.20	13.45	7.05	0.950	41.6	2.284
	Pith	100	0	0.005	0.3	4.6	7.2	62.2	26.0	1.194	63.0	21.90	58.70	14.50	4.90	0.695	36.7	1.894
	Roots	100	0	0.025	1.4	0.6	12.6	61.4	25.4	1.340	71.5	10.00	45.20	17.70	27.10	0.509	27.1	1.874

* For a more detailed description of plant parts see text.

TABLE VIII

Roots	100	0	0.025	1.4	0.3													

* For a more detailed description of plant parts see text.

TABLE VIII

MILLIGRAMS OF INORGANIC, SOLUBLE ORGANIC, INSOLUBLE ORGANIC, AND TOTAL NITROGEN PER GRAM OF FRESH WEIGHT IN THE DIFFERENT SECTIONS OF THE PLANT, TOGETHER WITH PERCENTAGE VALUES OF THE SAME AND OF THEIR FRACTIONS AS FOUND IN PINEAPPLE PLANTS GROWN IN SOLUTION CULTURES CONTAINING NITRATE NITROGEN

PLANT TISSUES	INORGANIC NITROGEN				SOLUBLE ORGANIC NITROGEN						INSOLUBLE ORGANIC NITROGEN						TOTAL N
	% OF INORGANIC		TOTAL	% OF TOTAL	% OF SOLUBLE ORGANIC				TOTAL	% OF TOTAL	% OF INSOLUBLE				TOTAL	% OF TOTAL	
	AMMONIUM	NITRATE			GLUTAMINE	ASPARAGINE	MONOAMINO	BASIC			AMIDE	MONOAMINO	BASIC	HUMINE			
	%	%	*mg.*	%	%	%	%	%	*mg.*	%	%	%	%	%	*mg.*	%	*mg.*
Leaves:*																	
Old B–1 (base)	17.0	83.0	0.029	2.7	18.30	18.30	30.70	32.70	0.290	25.30	8.25	67.50	6.06	18.17	0.825	72.0	1.144
2	65.0	35.0	0.017	1.5	20.90	22.40	27.60	29.10	0.254	22.20	6.78	80.40	4.59	8.27	0.871	76.3	1.142
3 (tip)	100.0	0.0	0.007	0.6	18.15	19.60	28.10	34.00	0.270	19.60	9.00	65.00	15.10	10.90	1.100	79.8	1.377
Mature C–1 (base)	3.0	97.0	0.219	10.6	13.30	14.40	26.90	45.40	0.368	29.20	9.67	68.80	8.78	12.80	0.672	60.2	1.259
2	10.0	90.0	0.062	5.8	18.07	18.08	27.70	36.15	0.321	29.90	9.85	71.30	6.38	12.46	0.690	64.3	1.073
3	64.0	36.0	0.011	0.8	19.66	15.94	26.45	37.95	0.295	20.70	7.72	62.80	17.40	12.20	1.116	78.5	1.422
4	100.0	0.0	0.005	0.1	20.40	17.60	21.50	40.50	0.284	15.40	7.63	62.90	19.70	9.85	1.559	84.5	1.848
5 (tip)	100.0	0.0	0.006	0.1	16.50	17.66	20.90	44.80	0.351	16.50	7.68	60.00	21.18	11.28	1.771	83.4	2.128
Active D–1 (base)	19.0	89.0	0.201	17.9	13.10	25.50	31.40	30.00	0.443	39.40	13.12	64.00	8.96	13.95	0.480	42.7	1.124
2	5.0	95.0	0.101	11.4	16.55	18.75	26.90	37.80	0.320	24.40	11.86	70.00	7.95	10.20	0.843	64.2	1.314
3	47.0	53.0	0.015	0.8	14.73	17.90	26.00	41.30	0.380	21.20	8.92	65.70	16.50	8.92	1.401	78.0	1.796
4	100.0	0.0	0.008	0.3	15.50	19.00	22.20	43.30	0.342	16.10	10.28	65.20	14.60	9.90	1.779	83.6	2.129
5 (tip)	100.0	0.0	0.008	0.3	2.63	6.77	40.00	50.60	0.310	13.00	13.27	61.10	16.65	9.04	2.071	86.7	2.389
Young E–1 (base)	9.0	91.0	0.069	.....	2.30	7.70	49.40	40.60	0.599	.....	.....	.....	.....	.....	.....	.....	.....
2	16.0	84.0	0.056	.....	3.10	7.90	50.00	39.00	0.354	.....	.....	.....	.....	.....	.....	.....	.....
3	60.0	40.0	0.015	.....	2.30	7.70	48.20	41.80	0.258	.....	.....	.....	.....	.....	.....	.....	.....
4 (tip)	100.0	0.0	0.010	.....	2.00	8.90	43.50	45.60	0.292	13.04	8.62	63.00	19.60	8.78	1.937	86.6	2.239
Stem:*																	
Tip B–Cortex	3.0	97.0	0.249	18.1	4.31	5.09	52.00	38.60	0.393	28.50	11.35	59.00	15.30	14.35	0.738	53.4	1.380
Pith	3.0	97.0	0.241	18.7	1.75	2.15	58.00	38.10	0.510	39.70	14.00	65.80	10.48	9.72	0.535	41.6	1.286
Middle C–Cortex	6.0	94.0	0.298	15.3	1.97	4.87	58.90	34.30	0.868	44.00	12.20	64.00	14.40	9.40	0.799	40.7	1.960
Pith	4.0	96.0	0.391	17.1	1.58	4.52	56.80	37.10	1.173	51.30	12.04	65.40	13.82	8.44	0.722	31.6	2.286
Base D–Cortex	2.4	97.6	0.320	15.8	2.93	7.00	53.00	37.15	0.956	47.00	11.75	64.40	17.75	6.10	0.755	37.2	2.031
Pith	7.0	93.0	0.506	18.1	2.10	10.10	54.25	33.50	1.275	46.00	12.70	64.30	16.60	6.40	0.996	35.9	2.777
Roots	2.8	97.2	0.772	28.0	2.10	4.25	56.50	37.15	0.611	22.00	12.00	66.00	10.15	11.75	1.387	50.0	2.770

* For a more detailed description of plant parts see text.

case the possible absence of certain substances essential for protein synthesis. It is difficult to explain the presence of comparatively great quantities of protein nitrogen in the presence of very small amounts of soluble organic nitrogen and of a large reserve of nitrate nitrogen. The authors' opinion is that such proteins are synthesized from soluble organic nitrogen translocated from the leaves through the stem and are probably not newly synthesized by the root tissues. Further studies are necessary to prove or disprove this contention. It is also possible that the great amounts of protein in the plants of the nitrate cultures are caused by a greater condensation of amino acids to protein under conditions of high sugar content. Total nitrogen values reported in tables VII and VIII are about 7.5 per cent. greater for the nitrate than for the ammonium-supplied plants, possibly on account of the accumulation of greater quantities of proteins.

STEMS.—The quantities of ammonium in the stems of the ammonium-supplied plants, as shown in figure 14-A, are exceedingly small and no nitrate is present. Explanations for both conditions have been offered in the section on roots and they apply equally well in the present case.

The values for soluble organic nitrogen are relatively high, indicating a high rate of translocation from the roots and a transient accumulation in the stem. It will be noted that the amounts of the different fractions are greater in the apical (D + E) than in the intermediate (C) and the basal (A + B) sections of the stem. It is possible that part of the soluble organic nitrogen fraction has been translocated to the stem from the leaves.

The relative amounts of protein (insoluble nitrogen) in the stem are comparatively moderate, but greater than in the roots and smaller than in the C and D groups of leaves. Carbohydrates or their derivatives are essential for protein synthesis from inorganic nitrogen and they are found in greater amounts in the stem than in the roots.

The amount of nitrate nitrogen in the stems of the plants of the nitrate-supplied series is comparatively high, according to the data shown in figure 14-B, which indicates a direct movement of this substance from the roots. It also indicates that its rate of assimilation in the stem is very low. Ammonium nitrogen is found only in traces in the various tissues, the possible sources of which have been already mentioned.

The amounts of soluble organic nitrogen in the stem are very great as compared to those in the roots and leaves. We cannot explain this condition in any other way except that the soluble fractions of organic nitrogen have been translocated from the leaves where nitrate nitrogen is assimilated very readily. Protein nitrogen in the stem is low when compared with that in the roots and leaves. The presence of great amounts of soluble organic and of small amounts of insoluble organic nitrogen indicate that the later steps in protein synthesis do not take place rapidly in the stem. Although

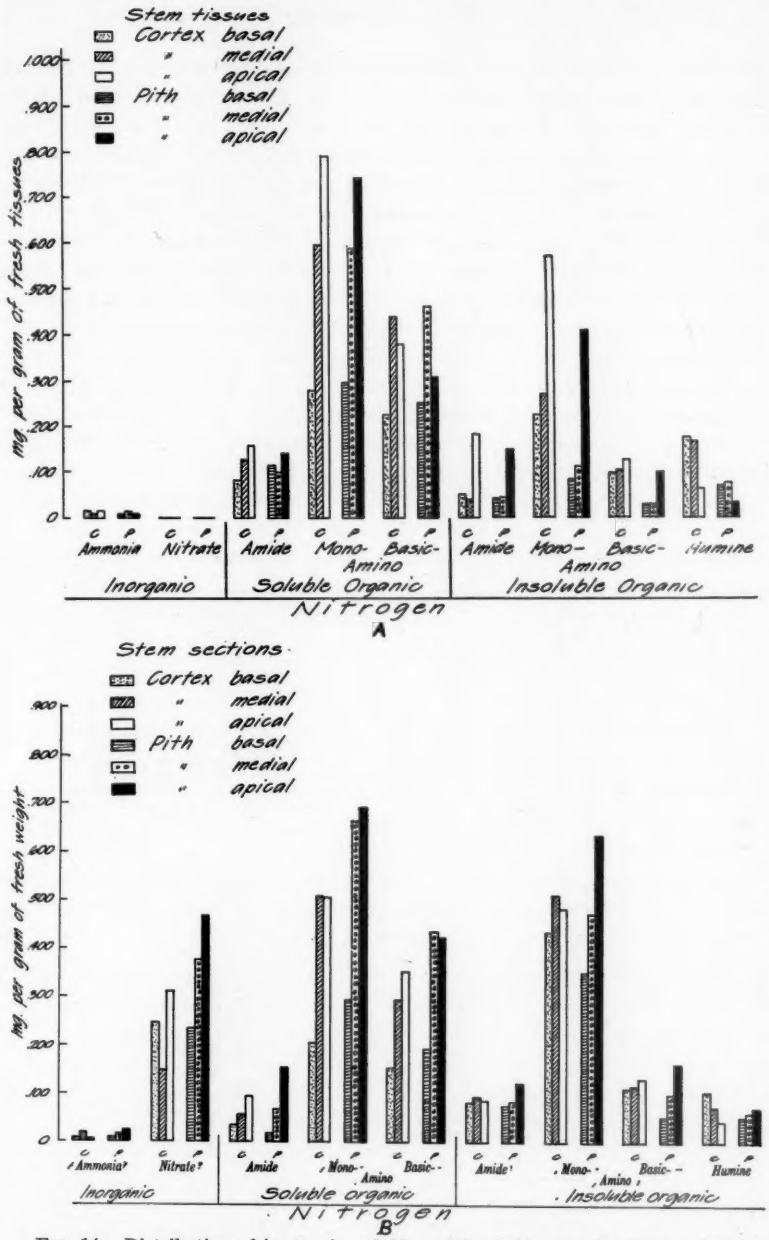

FIG. 14. Distribution of inorganic, soluble and insoluble organic nitrogen fractions in the various sections of the cortex and pith of the stem of plants with ammonium nutrition (A), and nitrate nutrition (B).

no satisfactory explanation can be offered, it may be pointed out that nitrate-supplied plants of many species display similar differences in the protein content of leaves, stems, and roots.

In a comparative study of the distribution of the soluble and insoluble fractions of organic nitrogen in the stem tissues, very little difference can be found between the plants of the ammonium and those of the nitrate series, possibly because of the position of the stem between roots and leaves, the two most active assimilating organs and sources of organic nitrogen. Total organic nitrogen as shown in tables VII and VIII and in figure 14 was consistently greater in the ammonium than in the nitrate-supplied plants.

LEAVES.—

Old leaves (group B).—This group of leaves represents planting-material-leaves which are composed of relatively old and partly senile tissues. The composition of the tissues of these leaves, therefore, cannot be compared with that of the other leaf-groups. The data in figure 15-A, show

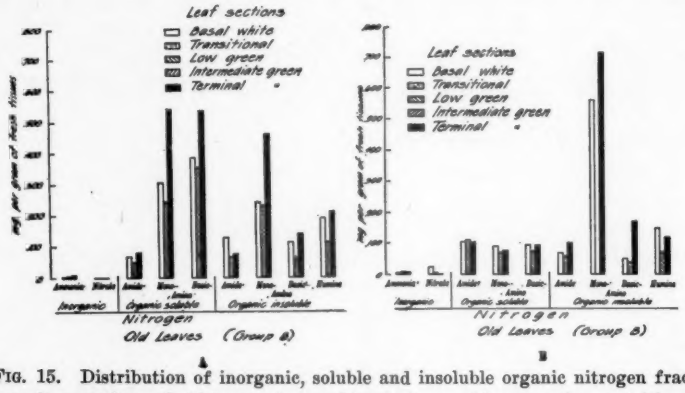

FIG. 15. Distribution of inorganic, soluble and insoluble organic nitrogen fractions in the various sections of old leaves (group B) of plants with ammonium nutrition (A), and nitrate nutrition (B).

the distribution of the different nitrogen fractions in the old leaves (group B) of the ammonium-supplied plants. There is indicated a very small concentration of ammonium and no nitrate in the various leaf sections. The amounts of soluble organic nitrogen are comparatively high, while those of protein or insoluble nitrogen are low. It is possible that the high values of soluble organic nitrogen represent translocated nitrogen which, owing to cessation of growth of the old leaves, is not utilized in the formation of new tissues; or it may represent decomposition products which are released from the proteins of senile tissues by hydrolysis.

The data presented in figure 15-B for the nitrate-supplied plants show

very small amounts of ammonium nitrogen in the white basal leaf tissue, but somewhat greater quantities of nitrate. Nitrate nitrogen has apparently entered the basal non-chlorophyllous tissues after passing through the roots and stem. However, it disappeared, presumably, as it penetrated into the chlorophyllous tissues.

Soluble organic nitrogen occurred in relatively small amounts in the old leaves of the nitrate-supplied plants. Amide nitrogen constituted a much greater portion of the soluble organic nitrogen fraction than mono-amino nitrogen. The latter was associated with plants of the ammonium rather than with those of the nitrate series, in the leaves concerned.

The concentration of protein nitrogen was, as in all the cases of nitrate nutrition so far studied, comparatively great. No statement can explain satisfactorily this condition in the absence of greater and more detailed data. Total organic nitrogen values were, on an average, 25 per cent. higher in the plants grown in the cultures with ammonium than in those with nitrate as figure 19 and tables VII and VIII show.

Mature leaves (group C).—This group represents the first leaves produced by pineapple plants after planting which in order of development are the oldest excepting those initially present on the planting-material piece. On plants five months old the number of such leaves is about 6, while in older plants it increases. The analytical data presented in figure 16-A show

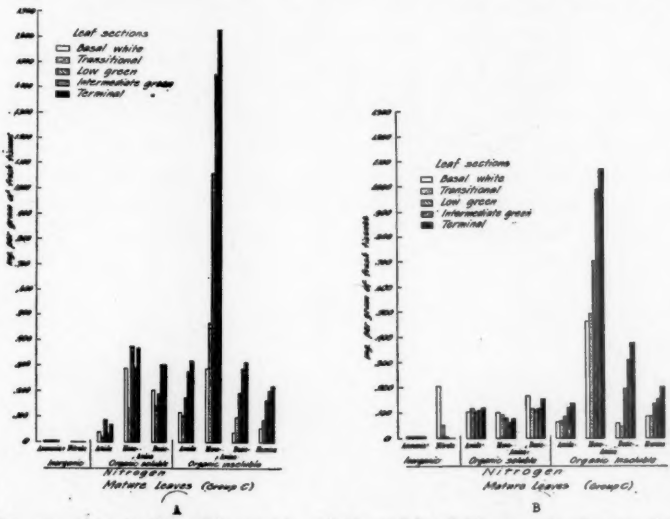

FIG. 16. Distribution of inorganic, soluble and insoluble organic nitrogen fractions in the various sections of mature leaves (group C) plants with ammonium nutrition (A), and nitrate nutrition (B).

that for ammonium-supplied plants, the amounts of ammonium nitrogen in the various sections of the old leaves were exceedingly small, while nitrate was entirely absent. Soluble organic nitrogen occurred in comparatively moderate concentrations with the mono-amino fraction present in greatest amounts. The insoluble organic nitrogen content was very high. The conditions favoring the synthesis of this fraction were probably very good in the green tissues of the old leaves where sugars, essential for protein synthesis, were present in sufficient quantities.

The quantities of ammonium nitrogen, as shown in figure 16-B, were very small for nitrate-supplied plants, whereas those of nitrate were exceedingly high in the basal, non-chlorophyllous tissues. The results obtained for inorganic nitrogen are similar to those for the older leaf group B except that the concentration of nitrate in the leaves of group C was considerably greater than in group B, owing probably to proportionally larger amounts of non-chlorophyllous tissues in the leaf bases of the group C plants and to more direct conduction, the latter point to be discussed later. The comparatively low plane of nitrate assimilation in the white basal tissue, a condition favoring the accumulation of nitrate in this region of the leaf, has been discussed previously. Soluble organic nitrogen occurred in relatively small amounts, with a trend of distribution of its different fractions similar to that in leaf group B.

Insoluble organic nitrogen in the old C leaves was very high and considerably higher than in the somewhat older B group of leaves. The conditions which favor the formation of great amounts of this form of nitrogen are an abundance of carbohydrates and certain other substances.

Comparative studies show that the amounts of insoluble organic nitrogen were greater in the leaves of the plants with ammonium than in those with nitrate nutrition as figure 16 shows. This condition was associated with a higher rate of absorption and assimilation of ammonium than of nitrate and also with the greater amounts of chlorophyll in the leaves of the plants with ammonium nutrition. Total organic nitrogen values, as reported in tables VII and VIII and figure 19 were, except for the basal white tissue, considerably higher in the plants supplied with ammonium than in those supplied with nitrate.

Active leaves (*group D*).—This group is composed of the longest leaves on the plant which are located between the fully grown and the more immature leaves. In four-months-old plants they number from 6 to 9 The analytical results for the various sections of the leaves of the ammonium-supplied plants are presented in figure 17-A. The quantities of ammonium were exceedingly small and nitrate was entirely lacking.

Soluble organic nitrogen occurred in relatively high concentration. The fractions, mono-amino and basic nitrogen, were considerably greater

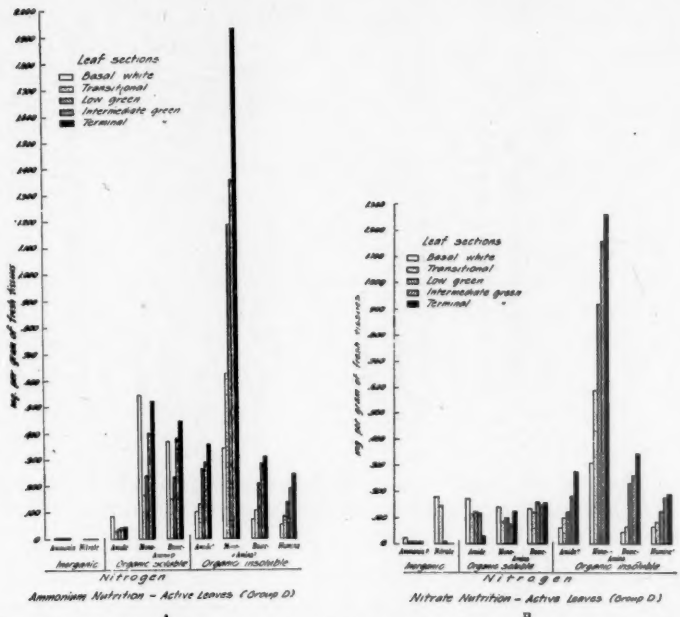

FIG. 17. Distribution of inorganic, soluble and insoluble organic nitrogen fractions in the various tissues of full grown active leaves (group D) of plants with ammonium nutrition (A), and nitrate nutrition (B).

than those of amide nitrogen. Insoluble organic nitrogen reached its greatest value in these leaves. Except for the accumulation of great amounts of protein the results are quite comparable with those of the somewhat older leaves of group C of the same series.

In the nitrate-supplied plants nitrate nitrogen occurred in comparatively great concentrations in the basal, non-chlorophyllous and in the transitional, sub-chlorophyllous tissues of the nearly fully expanded active leaves of the D group according to the data shown in figure 17-B. In the chlorophyllous tissues nitrate disappeared very readily, presumably owing to rapid synthesis to organic nitrogen. Ammonium nitrogen was present in small concentrations; its sources of derivation have been mentioned. The quantities of soluble organic nitrogen were moderate, the amide-nitrogen fraction being of approximately the same magnitude as the mono-amino and basic fractions. Protein content was very high, reaching its maximum concentration in the nitrate series in the D group of leaves.

Contrasting the protein content in the various sections of the large active leaves of group D of both series in figure 17, we find that in the ammonium

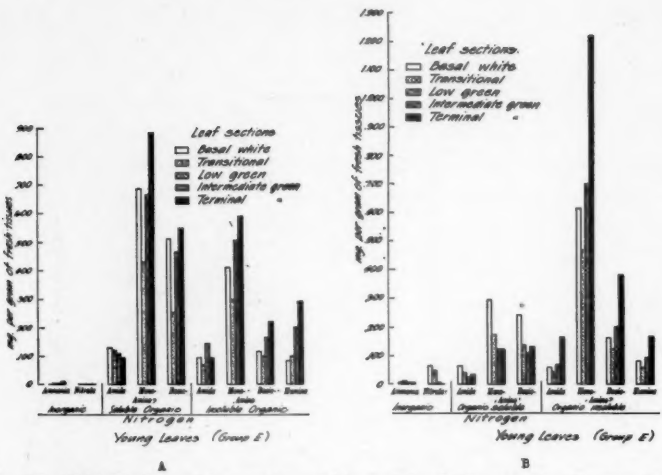

FIG. 18. Distribution of inorganic, soluble and insoluble organic nitrogen fractions in the various tissues of young leaves (group E) of plants with ammonium nutrition (A), and nitrate nutrition (B).

series the values are 30 per cent. higher than in the nitrate series. In this leaf group, as in the previous one, the causes for greater concentration of protein are a higher rate of ammonium than nitrate absorption, and concentrations of chlorophyll which possibly favored the synthesis of greater amounts of sugars in the plants of the ammonium than of the nitrate series. Total organic nitrogen values appearing in figure 19 and in tables VII and VIII were, on an average, about 27 per cent. higher in the ammonium than in the nitrate-supplied plants.

Young leaves (group E).—The leaves of this group are only partly developed, some having attained 80 and others 10 per cent. of their final growth. Associated with this condition the analytical data for ammonium-supplied plants, reported in figure 18-A show that the chemical composition of these leaves is, to a great extent, more like that of the immature (basal-non-chlorophyllous) tissues of leaf groups D and C than of the mature chlorophyllous tissues. Ammonium nitrogen was found in mere traces, except in the terminal tissues, where slightly greater amounts were observed, owing possibly to hydrolytic processes. Nitrate nitrogen, as in all cases of ammonium nutrition, was entirely lacking.

Soluble organic nitrogen and particularly the mono-amino and basic fractions were higher in these leaves than in any of the tissues so far studied.

Associated with proximity to chlorophyllous tissues, insoluble organic nitrogen was comparatively low, although higher than in the roots.

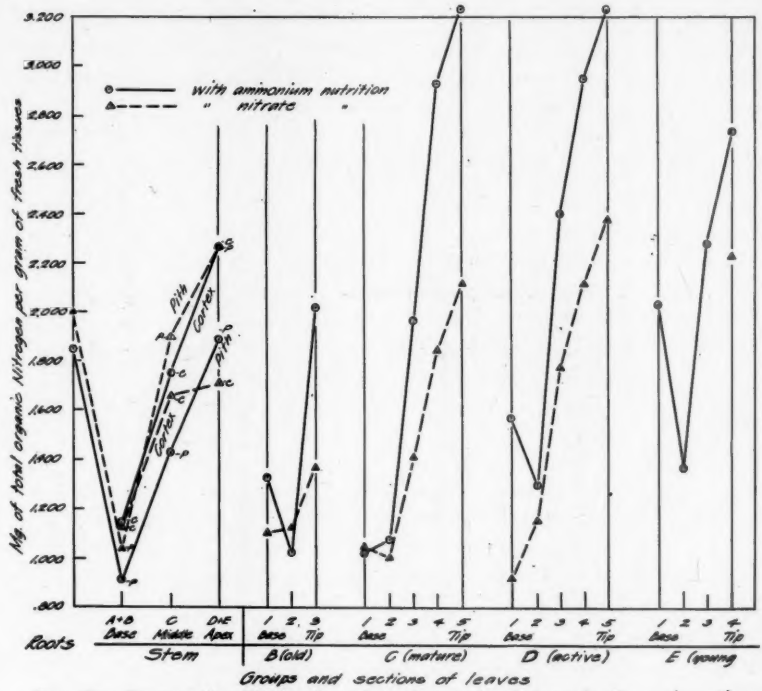

FIG. 19. Comparative distribution of total organic nitrogen in the various tissues of plants with either ammonium or nitrate nutrition.

According to the data shown in figure 18-B for nitrate-supplied plants, ammonium nitrogen was found in very small, while nitrate nitrogen was found in moderate, quantities. Of the soluble organic nitrogen fractions, glutamine was found in very small amounts while asparagine was found in appreciably greater ones. Mono-amino nitrogen values were significantly higher than in any other leaf groups of the same series, while those of basic nitrogen were approximately the same. The values for the insoluble nitrogen fractions are not complete on account of certain accidental losses of the samples during analysis.

Leaf section E-4 in the same figure shows greater amounts of insoluble nitrogen than the corresponding section of the ammonium series in figure 18-A.

DISTRIBUTION OF SUGARS

We are unable to report data on any other kind of carbohydrates than sugars, because the material to be used for analysis of starch, etc., was acci-

dentally subjected to comparatively high temperatures during dehydration and was found unfit for study. Sugars were determined in all cases on fresh tissue after thorough maceration in a brass mortar with quartz sand and subsequent extraction with ten volumes of water.

The distribution of reducing sugars and sucrose in the various sections of the leaves of the different groups as shown in table IX and figure 20

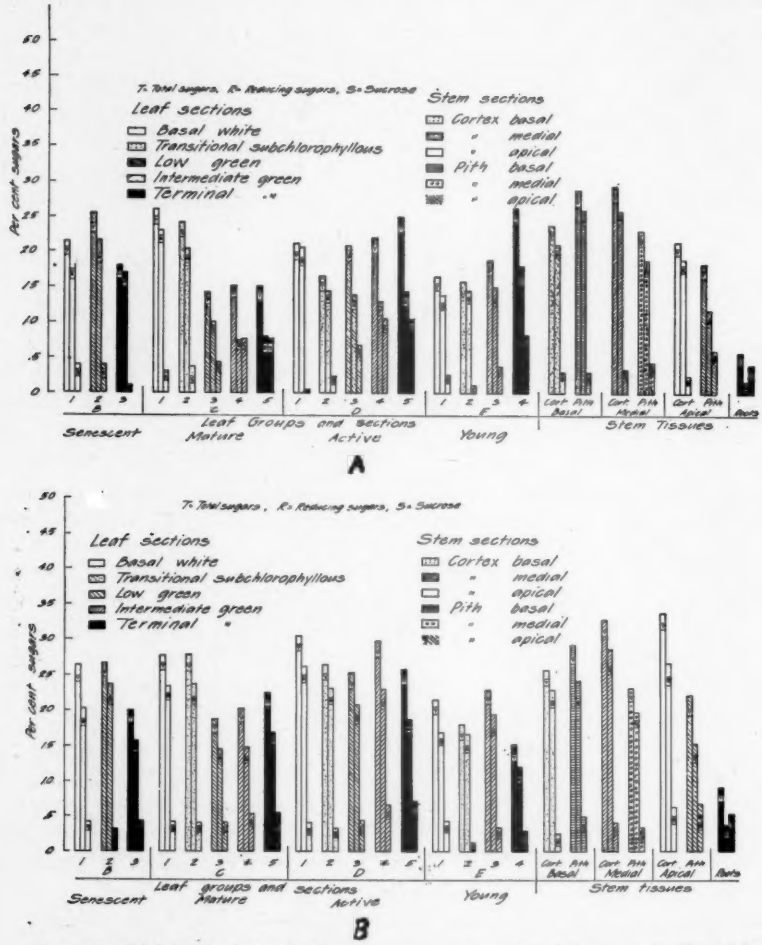

FIG. 20. Distribution of reducing sugars, sucrose, and total sugars in the various sections of the leaves of plants with ammonium nutrition (A), and nitrate nutrition (B).

differs considerably, but, with few exceptions, the order of distribution is practically the same for plants of the ammonium and nitrate series. Both kinds of sugars in the tissues of the old leaves of group B are irregularly distributed. This condition, owing to the presence of senile tissues, cannot be given serious consideration. In the sections of the mature group C leaves, reducing sugars and sucrose had definite order of distribution. The concentration of sucrose increased from the basal to the terminal tissue while reducing sugars decreased. In the nearly fully expanded active leaves of group D, the same order of distribution of both sugars was found as in the older C leaves. The concentration of reducing sugars in the terminal tissues of groups D and C were slightly higher than in the adjacent lower sections. The distribution of both reducing sugars and sucrose in the young leaves (group E) was somewhat different than in the two preceding groups of older leaves. The percentage of reducing sugars increased consistently from the basal to the terminal tissues. Sucrose, except in the basal tissues, followed the same order of distribution.

The order of distribution of sugars as observed in the chlorophyllous sections of the leaves of groups C and D was the same as that found in plants ranging in age from two to six months as observed in certain other studies (to be published later) where the distribution of sugars at different stages of plant growth was determined. In older plants the amounts of both reducing sugars and sucrose were higher in the chlorophyllous than in the subchlorophyllous and non-chlorophyllous tissues. Sucrose in plants of nearly all ages followed the same order of distribution, as has been found in the studies above mentioned.

In the tissues of the stem the order of distribution of reducing sugars decreased from base to apex in the pith, while in the cortex there was a tendency for the values to increase from the base until they reached the apex, where supposedly, they decreased on account of rapid utilization in new tissue formation. The distribution of total sugars in the tissues of the cortex of the stem is affected by translocation and the photosynthetic activity of the groups of leaves which serve directly the cells concerned. The concentration of sugars in the roots was comparatively very small. Sucrose was more abundant than reducing sugars. Not having direct evidence of the distribution of sucrose and reducing sugars in the stele and cortex of pineapple roots we cannot explain satisfactorily the difference in the quantities of these two sugars.

In somewhat related studies (**13**) conducted on the roots of *Pandanus veitchii* we found that the tissues of the cortex contained only reducing sugars and also that sucrose occurred exclusively in the tissues of the stele. As comparative data on the cortex and stele of pineapple roots are lacking we are unable to state definitely whether sucrose is exclusively restricted to

the tissues of the stele or whether it is distributed uniformly throughout the entire root. The presence of reserve or storage carbohydrate products, as sucrose, may explain at least in part, the rapid assimilation of ammonium and its conversion into soluble organic nitrogenous compounds.

The concentration of both reducing sugars and sucrose are from 10 to 25 per cent. greater in the plants of the nitrate than in those of the ammonium series, probably because of the relatively low rate of nitrogen assimilation. Both reducing sugars and sucrose are considerably lower in the roots of the plants of the ammonium than in those of the nitrate series. A condition probably associated with a greater rate of ammonium than of nitrate assimilation. The greater quantities of soluble organic nitrogen compounds in the roots of the plants with ammonium nutrition may account for the appreciably reduced values of sugars in the root tissues of this group of plants.

The difference in the distribution of sugars in the plants with ammonium and nitrate nutrition most worthy of consideration is that between the young tissues of the leaves, namely, the basal, non-chlorophyllous sections of leaf groups D and E and those of the apical section of the stem of the two respective series. These two groups of leaves, *viz.*, D and E, and the stem apex, not having attained complete development, are more actively engaged in growth processes and therefore more susceptible to biochemical variations than any of the other leaf groups or stem sections of the plant. The data in figure 20 show that the amounts of sugars in the basal non-chlorophyllous sections (no. 1) of the leaves and in the stem apex of the plants of the nitrate series are considerably greater than those of the corresponding sections of the ammonium series. These findings indicate that the rate of utilization of sugars in the sections of the leaves and stem under consideration was greater in the plants with ammonium than in those with nitrate nutrition. The distribution of sugars in the tissues of the terminal sections (nos. 4 and 5) of the E group of leaves is reversed. That is, the quantities of sugars in the terminal tissues of the leaves are greater in the plants with ammonium nutrition than in those with nitrate, indicating a greater rate of sugar utilization by the terminal leaf sections of the latter than of the former series of plants. The greater rate of sugar utilization by the terminal than the basal sections of the leaves of the plants with nitrate nutrition may be attributed to the synthesis of greater amounts of proteins and also of other nitrogenous substances such as amides and amino acids during and after the assimilation of nitrates.

The rapid utilization of sugars for the synthesis of soluble organic nitrogen compounds, as observed in the ammonium nutrition series, favors the formation of tissues of a high degree of succulence. Root tissues of a high degree of succulence, as obtained under conditions of ammonium nutrition.

are more susceptible to the ravages of parasitic pythiaceous fungi which may accidentally enter nutrient solution than the less succulent tissues of plants with nitrate nutrition.

Total dry organic values are the converse of the moisture content values of the tissues as shown in figure 10. They are, therefore, considerably greater in the nitrate than in the ammonium series of plants. Carbohydrates, representing about 90 per cent. of the constituents of dry organic matter, were produced in greater amounts in the nitrate than in the ammonium series of plants.

Discussion

Figures 12 to 19 show that the rate of absorption and the mode of assimilation of ammonium nitrogen was quite different from that of nitrate. Ammonium apparently was assimilated more or less completely in the root tissues, the determined products of its assimilation being large amounts of various amino acids and small amounts of glutamine and asparagine. These products accumulated in great quantities in tissues lacking chlorophyll, while in chlorophyllous tissues they were converted readily into proteins.

Nitrate nitrogen was neither absorbed nor assimilated as readily as ammonium. Nitrate, after absorption by the roots, moved through the conducting tissues of the roots and the stem into the leaves. In the basal non-chlorophyllous tissues it occurred in great amounts but as it entered the sub-chlorophyllous tissues of the transitional sub-chlorophyllous sections its concentration decreased and practically disappeared in the chlorophyllous tissues of the blade proper. These data indicate that nitrate was assimilated largely in the green tissues of the leaf. With nitrate nutrition the amounts of soluble organic nitrogen were relatively small except in the stem and in this organ possibly the soluble organic nitrogen was present in large part owing to translocation from the leaves. However, the quantities of protein were comparatively high in both chlorophyllous and non-chlorophyllous tissues, excepting the stem. On a percentage basis the findings indicated a greater rate of *protein* synthesis with nitrate than with ammonium nutrition in all tissues excepting the chlorophyllous ones of the mature C leaves and the nearly fully expanded D leaves of the ammonium series. This may well have been on account of their greater chlorophyll content and possibly greater sugar synthesis which, coupled with a higher rate of ammonium absorption as mentioned before, enabled these leaf sections to store greater quantities of protein.

With respect to the unequal distribution of soluble organic nitrogen in the non-chlorophyllous tissues, resulting either from ammonium or nitrate assimilation, consideration of certain anatomical features of the plant is advantageous. If we attempt to trace the different fractions of soluble

organic nitrogen from the tissues of the roots to those of the leaves of plants with ammonium nutrition we find that the amounts in the basal tissues of leaves decrease gradually from the youngest leaves (group E) to the old leaves (group B) as shown in figure 21-A. Assimilated products move

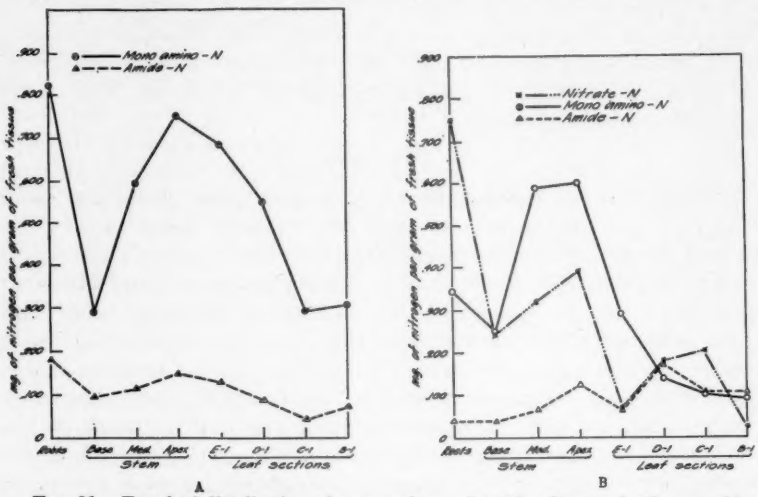

FIG. 21. Trend of distribution of monoamino and amide nitrogen in the non-chlorophyllous tissues of plants with ammonium nutrition (A), and nitrate nutrition (B).

through sieve tubes or conduits of similar physiological function, whereas non-assimilated ones move through tracheae or tracheids. The more recent theories on the movement of organic nitrogen and carbohydrates as expounded by CURTIS (1) and MASON *et al.* (6) claim that such substances may move either downward or upward depending on their location, origin, and ultimate physiological use. Translocation of soluble organic substances to meristematic tissues is generally known to be greater than to tissues which have ceased to grow. Therefore, the presence of greater amounts of soluble organic nitrogen in the younger than in the older tissues is to be expected from the point of view which has been presented.

According to conventional theory the movement of nitrate from roots to leaves is presumably conducted exclusively by the tracheae. In meristematic tissues, such as are in the apex of the stem, the tracheids are in an embryonic state and their capacity for conduction is very low. However, in the mature tissues such as leaves of groups B, C, and D the tracheids are well developed and their capacity for conduction is at a maximum. If we except the basal tissues of leaf group B on account of senility, we find that the amounts of nitrate in the basal leaf tissue of all the other groups are in a descending

scale from mature leaves (C) to young leaves (E) as shown in figures 21-B, which is in harmony with our theoretical expectations. Various other anatomical studies conducted by the senior author (5) have definitely shown that the relative length of individual vascular bundles varies consistently with their position in the stem and the relative length of the internodes. The vascular bundles of the stem that serve the basal tissues of the plant are considerably shorter than those serving the regions toward the apex. With the degeneration of the root primordia in the extreme basal tissue of the stem and the production of roots at higher levels, conduction of water and minerals is diverted by the longer fibrovascular bundles toward the leaves at higher levels on the stem axis. Clearly these observations are also in support of the mode of distribution of nitrate in the basal tissues of the leaves of the different groups as already presented.

Examination of the values in table VII, for ammonium-supplied plants shows that in tissues which have attained complete development but have not yet reached senility, the percentage values of soluble organic and protein nitrogen are practically constant. The actual amounts differ considerably. As an example of this condition there may be examined the percentage values of total soluble and insoluble organic nitrogen of the leaf sections 2 (transitional tissue), 3, 4, and 5 (tip) of mature leaf group C and of active nearly expanded leaf group D. The values for insoluble organic nitrogen range between 71 and 77 per cent., and those for soluble organic from 23 to 29 per cent. The small percentage variations are entirely insignificant when considered from the point of view of absolute variations which, in the case of insoluble organic nitrogen in sections 2, 3, 4, and 5 of leaf groups C and D, range from 0.768 to 2.818 mg. and in that of soluble organic nitrogen from 0.314 to 1.012 mg. With very old or very young leaves similar comparisons may be misleading for reasons stated previously. The sections of the stem showed very great variability, possibly on account of the presence of translocated products and great differences in the age of the tissues of the various sections.

Table VIII, dealing with the nitrate-supplied plants, shows somewhat different results from analogous plant parts. The proportionate values of insoluble organic nitrogen increased gradually from the basal tissue (2) to the tip (5) of the leaves while those of soluble organic nitrogen decreased in the leaves of groups C and D. A similar contrast between the proportional values for inorganic and soluble organic nitrogen showed that the values for both fractions decreased in the same direction. An analysis of the conditions as found in the various tissues shows that the entrance of inorganic (nitrate) was followed by the apparent synthesis of soluble organic nitrogen which was ultimately converted to insoluble organic nitrogen. With processes operating rhythmically, the final or ultimate products of synthesis,

the proteins, would accumulate in greatest amounts in the terminal tissues, and the first products of inorganic nitrogen assimilation, that is, those of soluble organic nitrogen, would be expected to be found in greatest concentration in the basal tissues. The differences in the proportionate distribution of soluble and insoluble organic nitrogen between the different leaf sections of plants with ammonium and nitrate nutrition are apparently caused, as explained above, by differences in their physiological function. The leaves of plants with nitrate nutrition changed inorganic into soluble organic nitrogen besides converting the latter into protein. In contrast, within the limits of nitrogen metabolism, the leaves of the plants of the ammonium series were concerned only with the latter process, as the first one was apparently performed by the roots. The direct inflow of soluble organic nitrogen into the leaves of plants with ammonium nutrition increased the percentage of protein nitrogen in the different sections at approximately the same rate. With nitrate-supplied plants the percentage rate of protein accumulation in the different sections was not constant, because of the direct inflow of unassimilated nitrogen (nitrate) which necessitated conversion first into soluble organic nitrogen and second into proteins.

The data with respect to the mode of assimilation of ammonium and nitrate nitrogen as contrasted in figure 22 show that the ratio between percentages of protein and soluble organic nitrogen is practically 3:1 in the different chlorophyllous leaf sections of the plants of the ammonium series regardless of great variations in the actual weights which are represented as mg./gm. of fresh tissues. Similar conditions are not obtainable in the chlorophyllous sections of the leaves of the plants of the nitrate series, because of the inflow of non-assimilated nitrogen (nitrate) which seemingly interferes with the rhythmical conversion of soluble organic nitrogen to protein. The ratio differences between percentage soluble organic nitrogen and protein of the chlorophyllous sections of plants with ammonium and nitrate nutrition may be employed for differentiating plants grown under the two different types of nutritional conditions.

The factors involved in the assimilation of nitrate have engaged the attention of many workers at different times. SCHIMPER (**10**) claimed that nitrates become depleted in the green leaves of plants exposed to daylight but that they were retained in those kept in the dark. WARBURG and NEGELEIN (**15**) and MOORE (**7**) have supported SCHIMPER'S findings. With more extensive studies, which have included a greater number of plant species, information has accumulated showing that different plants behave differently in their mode of nitrate assimilation. According to the information of more recent publications (**8**), many other plant organs besides leaves are able to assimilate nitrate both in light and in darkness if carbohydrate reserves are adequate.

The rate of nitrate assimilation by the chlorophyllous tissues of pineapple leaves is greater than that of the non-chlorophyllous tissues as indicated by the localization of nitrate and the products of nitrate reduction. Carbohydrates are highly abundant in all non-chlorophyllous tissues. Apparently in the pineapple plant external or other internal factors essential for nitrate assimilation are relatively more favorable in the chlorophyllous tissues.

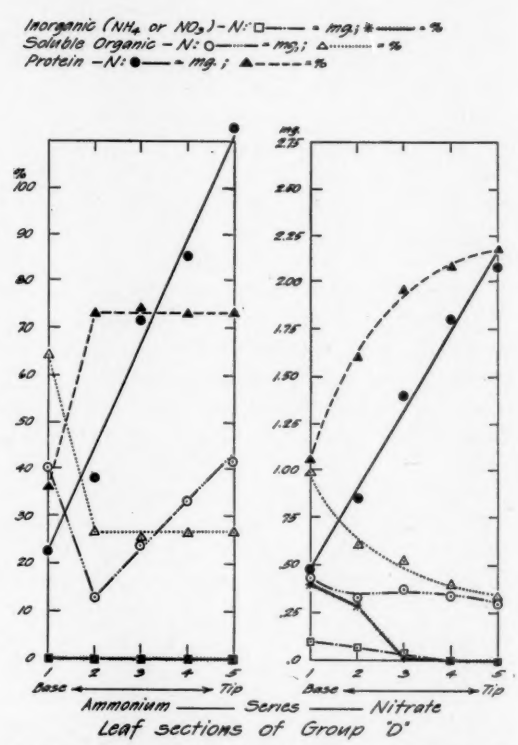

FIG. 22. Distribution of actual weight and percentage values of inorganic, soluble organic, and protein nitrogen in the different sections of the full grown active leaves (group D) of plants grown in ammonium (A) and nitrate (B) solution cultures.

In our earlier publication (13) we have discussed the conditions which, according to certain investigators, favor a higher rate of protein synthesis from nitrate than from ammonium nitrogen.

TABLE IX

DISTRIBUTION OF TOTAL AND REDUCING SUGARS AND SUCROSE IN DIFFERENT SECTIONS OF LEAVES, STEMS AND ROOTS OF PINEAPPLE PLANTS GROWN IN SOLUTION CULTURES SUPPLIED WITH EITHER AMMONIUM OR NITRATE SALTS AS SOURCES OF NITROGEN

Plant tissues	Ammonium cultures			Nitrate cultures		
	Reducing sugars	Sucrose	Total sugars	Reducing sugars	Sucrose	Total sugars
	%	%	%	%	%	%
Leaves:						
Old						
B1 (base)	1.74	0.39	2.13	2.20	0.42	2.62
B2	2.17	0.39	2.56	2.36	0.31	2.67
B3 (tip)	1.69	0.10	1.79	1.56	0.44	2.00
Mature						
C1 (base)	2.31	0.29	2.60	2.35	0.43	2.78
C2	2.04	0.38	2.42	2.36	0.43	2.79
C3	0.99	0.43	1.42	1.46	0.42	1.88
C4	0.75	0.76	1.51	1.49	0.54	2.03
C5 (tip)	0.81	0.77	1.58	1.70	0.55	2.25
Active						
D1 (base)	2.06	0.05	2.11	2.63	0.42	3.05
D2	1.44	0.22	1.66	2.32	0.34	2.66
D3	1.40	0.67	2.07	2.08	0.46	2.54
D4	1.30	0.89	2.19	2.31	0.67	2.98
D5 (tip)	1.45	1.05	2.50	1.88	0.71	2.59
Young						
E1 (base)	1.38	0.28	1.66	1.69	0.45	2.14
E2	1.46	0.11	1.57	1.67	0.14	1.81
E3	1.49	0.38	1.87	1.95	0.35	2.30
E4 (tip)	1.80	0.82	2.62	1.23	0.29	1.52
Stem:						
Base						
B–Cortex	2.08	0.30	2.28	2.30	0.26	2.56
Pith	2.61	0.29	2.90	2.42	0.50	2.92
Middle						
C–Cortex	2.61	0.33	2.94	2.86	0.41	3.27
Pith	1.87	0.42	2.29	1.98	0.34	2.32
Apex						
D–Cortex	1.90	0.26	2.16	2.69	0.63	3.32
Pith	1.21	0.61	1.82	1.54	0.68	2.22
Roots	0.18	0.39	0.57	0.38	0.54	0.92

Summary

This publication reports the results of experiments conducted under greenhouse conditions with pineapple plants grown in water cultures containing either nitrate or ammonium salts as sources of nitrogen. The objects of the investigations were to obtain data on growth, chlorophyll and moisture content of the different tissues, and distribution of different nitrogenous fractions, the latter to furnish information on the mode of assimilation of ammonium and nitrate nitrogen. The data obtained are presented in tables and figures, and may be summarized briefly as follows:

1. Light conditions were reasonably satisfactory and pH values between 4.2 and 5.5 were maintained as constantly as possible. Nitrogen was not greatly in excess of 28 p.p.m. and under these conditions pineapple plants grew approximately at the same rate when their nitrogen was supplied in the form of either ammonium or nitrate salts.

2. Ammonium nitrogen was absorbed from the nutrient solution at a considerably greater rate than nitrate, and was more rapidly assimilated than the former. The greater rate of ammonium absorption was responsible for the greater quantities of total nitrogen found in plants with ammonium nutrition, and may also account for the higher amounts of chlorophyll and the greater tissue succulence in these plants.

3. Ammonium nitrogen was apparently assimiliated in the roots as rapidly as it was absorbed; the immediate products of its assimilation were amino acids, and small quantities of glutamine and asparagine.

4. Nitrate nitrogen was assimilated very slowly, if at all, in the tissues of the roots. Most of it was translocated through the stem and the non-chlorophyllous tissues of the leaf bases presumably to the chlorophyllous tissues. Since little or no nitrate was found there, it would appear that in the chlorophyllous tissues it was converted very rapidly, first into various compounds of soluble organic, especially asparagine and basic amino, and then into protein nitrogen.

5. Plants with ammonium nutrition contained in their non-chlorophyllous tissues comparatively great amounts of soluble organic nitrogen and small quantities of protein. Plants with nitrate nutrition were somewhat different in this respect, the roots especially showed large quantities of protein as compared with those of soluble organic nitrogen.

6. The chlorophyllous sections of the mature C leaves and the active but nearly full grown D leaves of the plants with ammonium nutrition contained greater amounts of both soluble and protein nitrogen than those of the plants with nitrate nutrition. The chlorophyllous sections of leaf group B (old) and E (young) are excluded from this generalization, the former because they contained senile and the latter because they contained immature tissues.

0.92
0.54
0.38
0.57
0.39
0.18
Roots

7. The conditions responsible for the differential distribution of inorganic, soluble organic, and protein nitrogen in the corresponding parts of plants with ammonium or nitrate nutrition were caused, at least in part, by the method and rate of assimilation of the two forms of nitrogen.

8. The amounts of reducing sugars and sucrose were in general somewhat greater in the leaf tissues of the plants with nitrate than with ammonium nutrition, indicating a lower rate of carbohydrate utilization in nitrogen assimilation. The quantities of both kinds of sugars were considerably lower in the root tissues of the plants with ammonium than with nitrate nutrition. This condition, coupled with the presence of great amounts of compounds of soluble organic nitrogen, indicates that the sugars of the root tissues of the ammonium-supplied plants were utilized for the synthesis of these compounds from ammonium.

9. Values for the percentage distribution of soluble organic and protein nitrogen in the different sections of the leaves of groups C and D, presented in tables VII and VIII and in figure 22, are more constant for plants receiving the ammonium than the nitrate nutrition. The causes may be ascribed in the case of the plants with ammonium nutrition to the translocated soluble organic nitrogen compounds, which, under a wide variety of conditions, are readily converted to proteins. The irregular distribution of soluble and insoluble organic nitrogen values obtained in the leaves of the plants with nitrate nutrition were associated with the inflow of non-assimilated nitrogen (nitrate) which had first to be converted into compounds of soluble organic nitrogen and then secondly into proteins. Any factor or factors interfering with the first step in assimilation of nitrate might well result in the irregular values of soluble organic and protein nitrogen that were found to occur in the respective leaf sections of the different leaves of the nitrate-supplied plants.

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PINEAPPLE EXPERIMENT STATION
UNIVERSITY OF HAWAII

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ELECTRICAL POLARITY AND AUXIN TRANSPORT

W. G. CLARK

(WITH SIX FIGURES)

Introduction

The polar transport of indole-3-acetic acid (heteroauxin, β-indolyl-acetic acid) in the *Avena* coleoptile has been explained[1] on the basis of electrical polarity (**13, 15, 26, 42, 45**). The writer (**15**) has produced evidence showing that the electrical polarity in several plants is in the right direction to explain the basal, polar transport of the negative ion of heteroauxin in plants. He then showed (**14, 16**) that gravity, light, and applied potentials could reverse the inherent electrical polarity without affecting the polar transport. It was concluded that electrical polarity either has no cause and effect relation to the polarity of heteroauxin transport in the *Avena* coleoptile, or that this relation was not amenable to treatment by the various bioelectric methods utilized. The relation may be real,[2] but more subtle than could be revealed by the electrometric measurements employed.

It was suggested (**15**) that both bioelectric polarity and polar transport of heteroauxin might be due to polar permeabilities of individual cell membranes in the plant. GUHA (**18**) described a preferential conductance in the styles of *Narcissus, Aesculus,* and *Primula.* A platinum electrode was inserted into the ovary, another into the stigma, and a direct current was passed from a 2-volt source. The "conductivity" of the style was measured by deflections on a galvanometer in series with the plant and electrodes. Current passed more easily from stigma to style than in the reverse direction. Pollination abolished this "unipolar" conductance. GUHA'S work is difficult to judge reliably, as platinum electrodes develop large contact potentials very easily and would obscure real effects. Even if this criticism did not hold, GUHA was probably measuring differential polarization capacities rather than preferential conductance (**3, 4, 5, 6**).

METZNER (**30**) attempted to demonstrate a "polar conductivity" in the cutinized epidermis of fruits (apple, tomato, *Physalis, Clivia*); seed coats (*Pisum, Phaseolus, Aesculus*); parenchymatous tissue such as discs of the midrib of *Rheum* leaves; root of *Daucus;* and in the algae *Nitella* and *Chara.* He used these objects as rectifiers of an alternating current (A.C.) of 50 cycles, measuring the rectified direct current with a D.C. galvanometer in

[1] WENT and THIMANNS' recent book, Phytohormones (**45**) will be found to contain detailed discussions of most of the procedures and theories mentioned in the following pages.

[2] Sections of *Impatiens* hypocotyls have a bipolar staining capacity. Positively charged dyes are taken up most by basal cut surfaces, and negatively charged ones by apical cut surfaces (**15, 42**).

series with the calomel half-cells making contact with the tissue studied. No data are given.

The first part of the present paper is concerned with experiments performed to determine whether or not such a thing as unipolar conductance (unidirectional permeability) exists, and if so whether or not it can be correlated with unidirectional or polar transport of heteroauxin in the *Avena* coleoptile. The second part deals with the effect of ether narcosis on electrical polarity. VAN DER WEIJ (**40**) showed that ether narcosis reversibly abolished polarity, and BONNER (**7**) pointed out the dependence of polarity on respiration. Hence the effect of ether narcosis on the electrical polarity became a step in the writer's thesis that bioelectrical polarity, as it is ordinarily understood and measured, has no relation to polar transport. The third part of the paper will demonstrate the specific abolishment of polar transport without affecting electrical polarity, protoplasmic streaming, growth, respiration, or turgor. Independence of the mechanisms responsible for lateral transport in tropisms, and longitudinal transport of heteroauxin will be shown to exist.

Experimentation

UNIPOLAR CONDUCTANCE

It was shown in an earlier paper (**15**) that the potential differences (P.D.) of cut sections of coleoptiles were proportional to section lengths. In order to effectively magnify electrical phenomena in sections, it was found very convenient to pile sections one on the other in series. This was done also with the object in view of performing transport tests at the same time as the electrical measurements were made. Twenty-five 5-mm. sections of *Avena* coleoptiles were cut in the VAN DER WEIJ (**39**) section cutter, placed upright on a tap water agar block $11 \times 8 \times 1$ mm., a similar agar block being placed on top of the sections. Another twenty-five sections were placed on this block, and so on; so that finally four tiers, each consisting of twenty-five sections, were prepared. Tenth normal KCl agar strips made electrical contact to the top and bottom blocks of this preparation, the N/10 KCl agar leading to N/10 KCl solutions in cups outside of a moist chamber in which the preparation was situated. Calomel|saturated KCl electrodes were placed in these cups, and were used both for applying and measuring P.D. and current passage. Currents of the order used in these experiments were never found to polarize these electrodes. All nonconducting parts were covered with a thin coat of ceresin, and the chamber was darkened.

Several experiments were performed, in which *Pisum* stem-sections and *Avena* sections were used and the inherent P.D. was measured with a Wulf string electrometer (**15**). The resistance of the setup, with no sections present, was measured by applying a known voltage and measuring the direct

current passage by means of a calibrated galvanometer (sensitivity about 1-mm. deflection for 10^{-8} amperes at 1 meter scale distance, period 5 sec.). Knowing this resistance, the resistance of the sections could be computed by Ohm's law, from the electrometer measurements of the section P.D., and the galvanometer deflections caused by the current flow resulting from the section P.D. The changes in P.D. and resistance were then studied when (1) alternating current, (2) direct current, and (3) ether vapors were applied.

It was found, in *Avena* sections, that small direct currents (0.6 to 2×10^{-7} amp.) caused decreases in resistance essentially equal to those caused by larger D.C. (6×10^{-7} amp.). This decrease was, in one experiment, $35{,}000 \pm 5{,}000$ ohms for 0.6×10^{-7} amp., as well as for 6×10^{-7} amp. The P.D., however, changed only 1 or 2 millivolts (mv.) when 10 mv. (0.6×10^{-7} amp.) were applied (fig. 1), while it changed 15 to 20 mv. when 100 mv. (6×10^{-7} amp.) were applied. In this experiment (fig. 1), a typical case, the morphological bases were, as usual, electropositive to the tips (**15**). When 10 mv. were applied, in series with the section P.D., the latter dropped 3 mv.; when applied in parallel with the section P.D., no perceptible change resulted. Such a difference is within the limits of individual variation in the determinations. Fifty mv. in series, decreased the inherent P.D. approximately 10 mv., and when in parallel, increased it about 7 mv. Thus in the cases of 3 to 50 mv. applied P.D., the direction of flow of applied current had no real difference in effect on the inherent P.D. But 100 mv., in series, decreased the section P.D. 15 mv., reversing the inherent electrical polarity; and, when in parallel, increased it 20 mv. The time relations of these changes are shown in figure 1. The return (recovery, depolarization) curves show that the processes affected by applied P.D. are reversible. The normal polarity is quickly restored when the applied P.D. is removed. In an earlier paper (**16**) on applied P.D. and auxin transport, longer periods (5 to 60 or 120 minutes) of current application were used. In those experiments, polarization is more permanent, and depolarization occurs much more slowly. This recalls similar results described by MARSH (**29**) and by BLINKS (**3, 4, 5**).

The important conclusion to these observations, so far as the present thesis is concerned, is that the resistance drops when P.D.'s are applied, regardless of the polarity of the applied P.D. This presumably means that the protoplasmic surfaces are altered so that current is carried more easily by ions, *i.e.*, that permeability has increased. The absence of polar effects of the applied P.D. (the differences are within the limits of individual variability) do not confirm the results of MARSH (**29**) or BLINKS (**3, 4, 5**), but it is frankly admitted that no attempts were made to study this phase of the problem, and more careful observations may reveal such a characteristic. The sensitivity of the resistance-measuring apparatus was really not high enough to link resistance changes with P.D. changes as quantitatively as could be de-

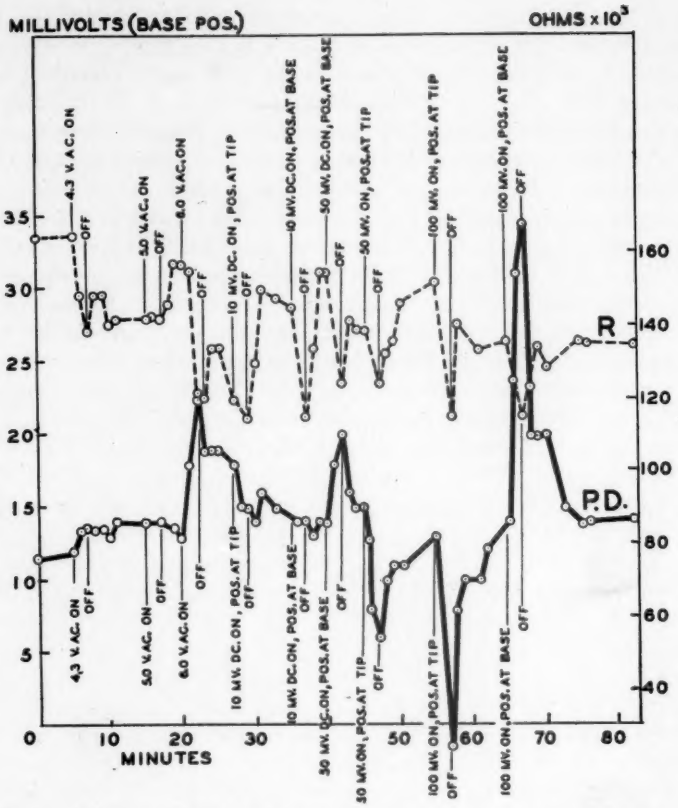

FIG. 1. Effect of applied direct and alternating currents on inherent P.D. and R of *Avena* coleoptiles.

sired, but it is clear enough that the resistance drops just as much with 10 mv. applied P.D. as with 100 mv. (or with 6 volts A.C.).

It would be highly desirable to study more closely the resistance and potential changes with applied P.D., especially since BLINKS (**5**) and OSTERHOUT (**32**) have shown that compound membrane potentials may be separated into simpler component P.D.'s by this method; or, in other words, it might be possible to analyze differential membrane permeabilities into simpler components. This may prove to be the only way to attack the electrical phenomena postulated to be linked with polar transport (**42**), ion accumulation (**21**), osmotic work performed in intestinal absorption (**24**) and in the kidney (**27**), etc., since mere measurements of normal inherent P.D.'s appar-

ently mean little, as has been shown in two former papers by the writer (**15, 16**), and as will be further proved in this paper.

Figure 2 represents the electrical behavior of coleoptile sections which happened to have had an inverted polarity which was slowly righting itself ("handling reaction," **15**). In this experiment, the sections were not piled in tiers. Coleoptiles were decapitated, the primary leaves removed, and the whole coleoptile removed from the rest of the seedling by cutting it just above the coleoptilar node. Forty such whole coleoptiles were placed in parallel, the P.D. between their cut surfaces being measured in the usual fashion.

It is seen in figure 2 that the resistance follows the P.D. Electronegativity decreases as the resistance decreases. The resistance was much lower than in the experiment depicted in figure 1, and hence the measurements were more accurate because galvanometer deflections were greater.

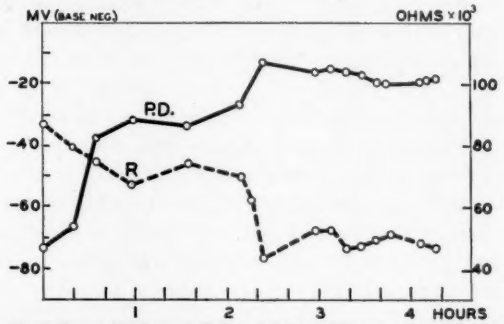

FIG. 2. Variation of inherent P.D. and R of *Avena* coleoptiles with time.

Desiring to see if an A.C. rectifying effect exists in the sections such as postulated by METZNER (**30**), A.C. (60 cycles) was applied to the sections for 1 to 2 minutes. Figure 3 shows that 3 volts A.C. has little or no effect on the P.D. or resistance (R), whereas 5 v. A.C. reversibly decreases R and increases the electronegativity of the apical surface (*cf.* effect of ether narcosis). This confirms the results of AMLONG and BÜNNING (**1**) and BÜNNING (**12**), who clearly demonstrated a correlation between permeability and bioelectric potentials. The permeability is altered only after a certain threshold of A.C. stimulation is reached (liminal threshold), after which some change in the surface properties of membranes brings about a sudden increase in permeability, and hence a drop in R and a change in P.D. on account of concentration changes and differential ion mobilities.

If one would believe METZNER (**30**), one would think that the D.C. deflections caused by the applied A.C. meant rectification of the A.C. by the plant tissues. This is an entirely erroneous conclusion, as shown by the P.D.

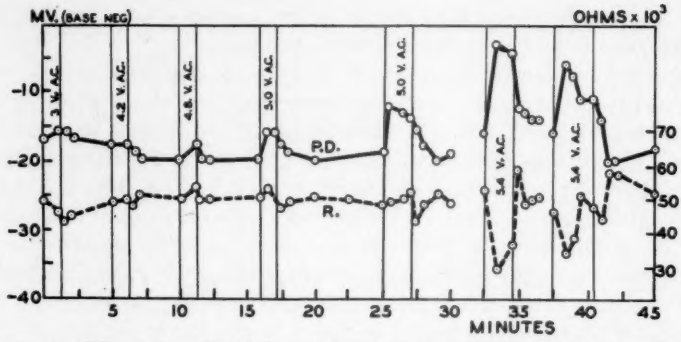

FIG. 3. Effect of applied A.C. on inherent P.D. and R of *Avena* coleoptiles.

measurements. No galvanometer deflection occurs unless the plant P.D. is altered, and hence the galvanometer deflection is caused by stimulation (polarization or permeability change) and not by ohmic rectification of the A.C.

It is seen in figures 1 and 3 that A.C. stimulation increases the negativity of the apical end of the sections (compare also reversible ether narcosis). This may mean that the apex is more easily stimulated or polarized than the base, with subsequent greater increases in permeability or polarization, and with resulting greater apical negativity. Increased permeability (caused by induction shocks) gives rise to negativity of the stimulated portion, as shown by AMLONG and BÜNNING (**1**).

EFFECT OF ETHER NARCOSIS ON ELECTRICAL POLARITY

Ether narcosis irreversibly abolishes electrical polarity if the narcosis results in complete loss of turgor. Figure 4 shows the drop in resistance and P.D. when air, saturated with water vapor, was bubbled through ether and aspirated through the experimental chamber containing the sections which were piled in series as described earlier. After 40 minutes, a residual apical negativity remains which probably represents the geoelectric effect found by BRAUNER and AMLONG (**11**), and discussed by WENT and THIMANN (**45**). Figure 4 also shows that 6 v. (A.C.) does not alter the P.D. in the completely narcotized tissue. The D.C. galvanometer, however, showed some deflection. Here, therefore, one might think that METZNER's unipolar conductance is being manifested in a rectification of the A.C. BRAUNER (**10**) demonstrated a unipolar permeability in certain dead plant tissues (seed coats), but did not determine whether such objects could rectify an A.C. In the narcotized sections, it is more probable that the applied A.C. merely increases permeability a bit more, and hence the R drops a little. The reason

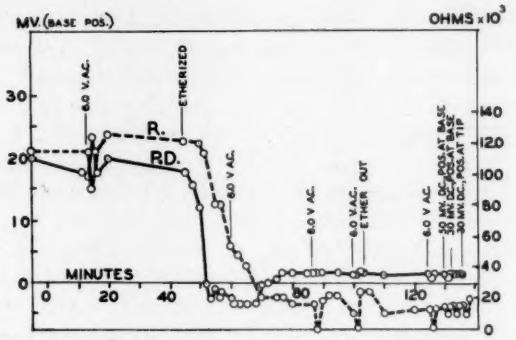

FIG. 4. Effect of irreversible ether narcosis on P.D. and R of *Avena* coleoptiles.

why no further P.D. changes result probably rests on the possibility that the narcosis has irreversibly destroyed selective ion permeability. No ohmic unipolar conductance exists in the narcotized tissue, as shown by equal galvanometer deflections, regardless of the polarity of applied small D.C. (*ca.* 10^{-7} amp.).

Reversible ether narcosis was obtained in the sections with inverted electrical polarity by passing air through water half saturated with ether at 20° C. and then aspirated through the experimental chamber. As seen in figure 5, the apical negativity was first increased for a brief period, then decreased, just as in complete narcosis. The R changes in the opposite way. The cause of the opposite changes in R in reversible and in irreversible narcosis is not known, as not enough experiments of this nature were performed, but it is well known that small concentrations of ether stimulate such processes as respiration instead of inhibiting them. Reversible narcosis with half-

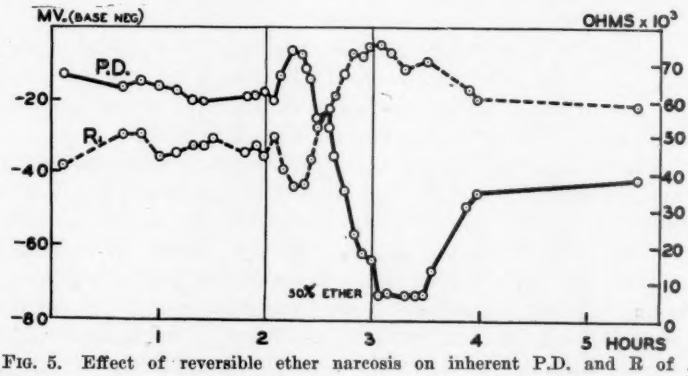

FIG. 5. Effect of reversible ether narcosis on inherent P.D. and R of *Avena* coleoptiles.

saturated ether increases the R of the sections, and decreases their apical negativity. Complete, irreversible narcosis decreases both. Hence the possibility remains that the increased resistance during reversible narcosis signifies a decreased permeability. This decreased permeability may be causally related to decreased polar transport of auxin. VAN DER WEIJ (**40**), as already mentioned, reversibly abolished polar transport by ether narcosis accomplished by narcotizing coleoptile sections by ether vapors from water half saturated with ether. Approximately this same concentration was used in the reversible narcosis depicted graphically in figure 5. Owing to lack of further experimental data, no further statements can be made at this point, but it is pointed out that here, for the first time, we have been given a clue to the physical properties of the mechanisms responsible for polar transport. It is hoped that further advances in this direction will be made.

EFFECT OF CERTAIN LYSINS ON HETEROAUXIN TRANSPORT

SPECIFIC ABOLISHMENT OF POLAR TRANSPORT OF HETEROAUXIN.—It has been shown in the second paper of this series (**16**) that the longitudinal transport of heteroauxin is independent of measured electrical polarity (apical negativity). This section will show that transport may be specifically abolished without apparently altering any other properties yet investigated. Some of these properties were semi-permeability, cell elongation, protoplasmic streaming, respiration, and electrical polarity. Thus an interesting and promising tool is offered for further investigations into the mechanism responsible for polar transport.

PONDER and MACLEOD (**35**) showed that the P.D. across a frog skin could be completely abolished by certain lysins, without affecting respiration. It was thought, therefore, that such substances might abolish the electrical polarity of *Avena* coleoptiles without affecting polar transport, since the latter is dependent upon respiration (**7, 40**).

It was entirely unexpected, therefore, when the results showed just the opposite. The lytic substances abolished transport, but had no effect on electrical polarity, nor, indeed, upon any other observed properties until the concentrations of lysin were high enough to be very toxic.

One way of testing the transportability of various auxins is to see if they will cause curvatures in *Avena* when applied in agar blocks on one side of decapitated coleoptiles. If no curvature ensues, and yet the substance is active in promoting growth, *e.g.*, as in the pea test (**19, 45**), the substance is not longitudinally transportable. For this reason, the lysins chosen for these experiments, saponin and sodium glycocholate, were applied in agar blocks to the decapitated apices of *Avena* coleoptiles for 3.5 hours, after which a second decapitation was made in which about 1 mm. of the tip was removed. Heteroauxin in agar blocks was then applied unilaterally to the decapitated

stump, the resulting curvatures being recorded 90 minutes after the heteroauxin application. The control curvatures averaged 15°. Table I presents the results. Twelve plants per test were used. (For further details concerning the *Avena* test refer to WENT and THIMANN, **45**).

TABLE I

EFFECT OF SAPONIN AND SODIUM GLYCOCHOLATE ON *Avena* TESTS WITH HETEROAUXIN

INFILTRATES IN AGAR BLOCK PLACED ON DECAPITATED COLEOPTILES 3 HR.	CURVATURES*	MEAN ERROR
	degrees	
Tap H_2O (control)	15.0	±1.8
Sodium glycocholate, 1: 100,000	11.8	±1.0
Sodium glycocholate, 1: 10,000	10.4	±0.7
Sodium glycocholate, 1: 1000	12.4	±1.5
Sodium glycocholate, 1: 50	12.0	±1.6
Tap H_2O (control)	12.3	±1.5
Saponin, 1 part saturated: 1000	13.0	±1.4
Saponin, 1 part saturated : 100	11.3	±1.8
Saponin, 1 part saturated : 10	15.3	±1.5

* The "maximum angle" (WENT and THIMANN, 45) was approximately 20° at the time of these analyses.

Considering the mean errors $\left(\sqrt{\frac{\Sigma(d^2)}{n(n-1)}}\right)$, where d = deviation from mean, and n = number of test plants (**2**), it is clear that no inhibition of *Avena* curvatures was obtained. The conclusion was tentatively reached that the large glucoside molecule, saponin, and the sterol-amino molecule, sodium glycocholate, did not enter the coleoptiles during the pretreatment before heteroauxin was applied.

For this reason, direct transport tests were made in which the coleoptile sections were infiltrated with saponin and sodium glycocholate, with a water-infiltrated lot for controls. Four-mm. sections were cut with the VAN DER WEIJ (**39**) section cutter, and placed with their hollow centers on the teeth of composition combs, to enable the experimenter to determine their morphological polarity after treatment in various solutions. The combs containing the sections were placed in sodium glycocholate and saponin solutions, and in water, and infiltrated for 2 minutes by releasing negative pressure produced in a desiccator. The combs were then removed, the solutions carefully blown from the hollow centers of the sections, the sections blotted, and six of them placed with their morphological bases downward on tap water agar blocks. Agar blocks containing 0.98 mg. per l. indole-3-acetic acid were placed on the apices. After 2 hours the bottom blocks were removed, cut into 12 smaller blocks, and tested by the *Avena* test, using the double decapitation method which included 3 hr. between decapitations and 90 min. between time of putting the agar blocks on the test plants and photographing them (**37**).

TABLE II

EFFECT OF SODIUM GLYCOCHOLATE AND SAPONIN ON HETEROAUXIN TRANSPORT IN COLEOPTILE SECTIONS

CONCENTRATION TEST (0.073 MG./L.) ..... 8.4°
7.3
Av. 7.8

7.8° = 1/40 STOCK SOL. (2.98 MG./L.), HENCE STOCK = 7.8 × 40 = 312°
TOP BLOCKS IN TRANSPORT TESTS = ⅓ STOCK = 312/3 = 104°
TRANSPORT TIME 2 HR.
6 4-MM. SECTIONS PER TEST

INFILTRATE IN SECTIONS	CURVATURE (AUXIN TRANSPORTED INTO BOTTOM BLOCKS)	MEAN ERROR	PROTOPLASMIC STREAMING	APPEARANCE OF SECTIONS
	degrees			
Sodium glycocholate, 1: 100	None	.....	–	Flaccid
Sodium glycocholate, 1: 500	None	.....	+	Turgid
Saponin, 1 part saturated: 20 ...	10.4	±1.4	–	Turgid
Saponin, 1 part saturated: 100 ...	11.1	±1.3	+	Turgid
H_2O	10.2	±1.1	+	Turgid

Table II shows the results. One column of this table shows the result of a microscopic examination for protoplasmic streaming in the sections after the transport tests had been made. The sections showed protoplasmic streaming when placed intact in a drop of water on a slide, or when cut into two halves with a razor blade and examined under water with white light and under 900 magnifications. Another column of the table describes the macroscopic condition of the sections.

The following conclusions can be formulated from the results given in table II: (1) Sodium glycoholate is toxic in a concentration of 1:100. It is not toxic in 1:500 for the time used. The criteria for toxicity were taken to be cessation of protoplasmic streaming, and the occurrence of flaccidity caused by loss of semipermeability and turgor; (2) Sodium glycocholate in nontoxic concentrations completely abolishes heteroauxin transport. This is not due to cessation of protoplasmic streaming; (3) Saponin, in concentration of 0.05-saturated, stopped protoplasmic streaming but had no effect on transport. This is very interesting, in view of the fact that WENT (**41**) and BOTTELIER (**9**) suggested that protoplasmic streaming may limit transport. SCHUMACHER (**36**), however, has shown that the transport of fluorescein in stem hairs of *Cucurbita pepo* is independent of protoplasmic streaming. Additional data concerning this relationship are needed. Saponin in higher dilution had no effect on heteroauxin transport, turgor, or protoplasmic streaming.

It is conceivable that the effect of sodium glycocholate may not be limited to the membrane mechanisms responsible for polar transport. In higher dilution (1:500) it does not obviously abolish semipermeability, as judged

by the retention of turgor; nor does it affect protoplasmic streaming. It might, however, inhibit some other mechanism which limits transport, for example, cell oxidations, and presumably, therefore, the sources of energy for the performance of concentration work in polar transport (*cf.* HOAGLAND and BROYER, **21,** HUF, **22, 23,** and VAN DER WEIJ, **40**), who show that concentration work depends on respiratory metabolism of the living membrane). BONNER (**7**) has shown that growth by cell elongation in the *Avena* coleoptile is limited by respiration. If, therefore, sodium glycocholate affects respiration, it should affect growth. Since, however, growth in intact *Avena* coleoptiles is limited by transport,[3] the effect of sodium glycocholate on growth must, on first thought, be studied by means of the pea test (**34, 43, 45**). In this test, polar transport does not limit growth. Hence the effects of sodium glycocholate and saponin on heteroauxin curvatures in the pea test were investigated. Etiolated *Pisum* seedlings were used about 7 days after soaking and planting. The first leaves were removed, and the apical end of the stem was split longitudinally for about 5 cm. The stem was then cut off just below the split part, and the split stems put into the solutions to be tested. Heteroauxin enters the intact sides of the split halves more than on the cut sides (**34**), increasing growth there, so that the two halves curl inward. The extent of this curvature is proportional to the activity of the heteroauxin on cell elongation.

The split pea stems were put into heteroauxin solutions containing saponin and sodium glycocholate. The curvatures were photographed after 3, 6, and 11 hours, the resulting curvatures being measured and plotted in figure 6. The concentration of heteroauxin used was 1.96 mg. per liter. Seven tests per solution were made.

It is seen that both sodium glycocholate and saponin in concentrations which do not cause a decrease in turgor, not only do not inhibit growth, but actually increase the final curvatures between 50 and 60 per cent. higher than the heteroauxin controls. Sodium glycocholate alone had a very small activity on the pea test, and saponin alone had none. The method of the facilitation of heteroauxin activity by these lytic substances is not yet known.

The important conclusion for the purposes of this discussion is, however, that apparently nontoxic concentrations of lysins do not inhibit the action of heteroauxin on plant growth, while one of them (sodium glycocholate) completely abolishes polar transport. This conclusion has been justified by repetition of the above experiments, using sodium glycocholate in concentrations as small as 1 : 100,000. The following experiment illustrates this justification:

[3] See section below in which the effect of sodium glycocholate on the growth of cut sections of coleoptiles shows that transport does not limit the growth of cut sections. This does not agree with BONNER'S statement that transport in sections limits growth, as shown by inhibition by lack of oxygen.

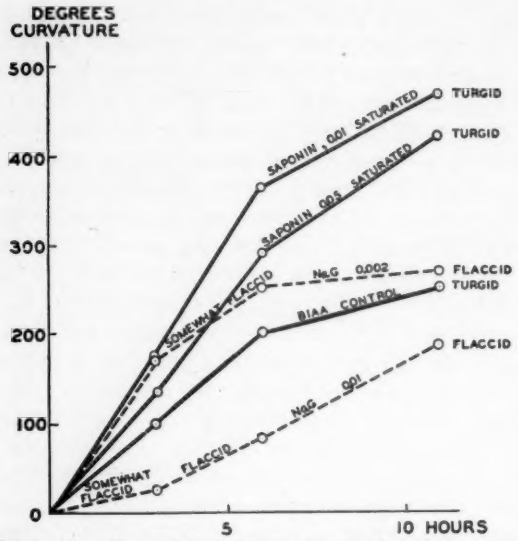

FIG. 6. Effect of sodium glycocholate and saponin on pea stems tested with heteroauxin (BIAA).

Four-mm. coleoptile sections were infiltrated, blotted, and a 2-hour transport test was performed with heteroauxin of a concentration of 356° in the apical blocks of six upright sections. The basal blocks were tested by the *Avena* test, using the double decapitation method. The sections were infiltrated with aqueous sodium glycocholate solutions of the concentrations indicated in table III.

The conclusion is that sodium glycocholate abolishes transport in very high dilution (dilutions higher than 1 : 100,000 have not as yet been tested), although protoplasmic streaming and semipermeability are unaffected.

TABLE III

EFFECT OF SODIUM GLYCOCHOLATE ON HETEROAUXIN TRANSPORT IN COLEOPTILE SECTIONS

INFILTRATE IN SECTIONS	ANALYSIS OF BOTTOM BLOCK (CURVATURE)	MEAN ERROR	PROTOPLASMIC STREAMING	APPEARANCE
	degrees			
H_2O	11.4	±1.4	+	Turgid
Sodium glycocholate, 1: 100,000	None	.....	+	"
Sodium glycocholate, 1: 10,000	"	.....	+	"
Sodium glycocholate, 1: 5000	"	.....	+	"
Sodium glycocholate, 1: 1000	"	.....	−	"
Sodium glycocholate, 1: 500	"	.....	−	Flaccid

As stated above, PONDER and MACLEOD (35) found that respiration was unaffected in frog skin, although the P.D. was abolished by 1:100 saponin and sodium glycocholate. It was shown above that heteroauxin transport is presumably specifically abolished.

The following experiment shows the effect of sodium glycocholate on the electrical polarity of coleoptile sections from the same lot that was infiltrated for the transport tests. The sections were placed in tiers to give series summation of P.D. Two tiers of ten sections each were piled up, and the P.D. between morphological tips and bases measured with the electrometer. Normal polarity existed, since the bases were electro-positive. Table IV shows the results of measurements 2 hours after setting up (about the same time that the transport tests were ended).

TABLE IV

EFFECT OF SODIUM GLYCOCHOLATE ON ELECTRICAL POLARITY OF COLEOPTILE SECTIONS

INFILTRATE IN SECTIONS	P.D. (BASE ELECTROPOSITIVE)	PROTOPLASMIC STREAMING	APPEARANCE
	mv		
H_2O	10	+	Turgid
Sodium glycocholate, 1: 100,000	10	+	"
Sodium glycocholate, 1: 10,000	10	+	"
Sodium glycocholate, 1: 5000	5	+	Less turgid
Sodium glycocholate, 1: 1000	0	−	Flaccid
Sodium glycocholate, 1: 500	0	−	"

The unexpected conclusion was reached that although 1:100,000 down to 1:5000 sodium glycocholate completely abolishes heteroauxin transport during the 2-hour transport test, it has no effect on electrical polarity (or protoplasmic streaming and semipermeability).

Pea tests confirmed the earlier observation that sodium glycocholate increases the activity of heteroauxin. In this test, unlike the former, the split pea stems were first infiltrated with the sodium glycocholate solutions before being placed in the sodium glycocholate-heteroauxin solutions. Such infiltration of the pea stems thus makes their physiological state more comparable with that of the infiltrated coleoptile sections. The pea curvatures were measured after 12 and 24 hours. The concentration of heteroauxin used was 0.73 mg. per liter. Ten tests were made in each solution. Table V shows the results:

The same facilitation by sodium glycoholate (NaG) of the final curvature reached in heteroauxin solutions is thus confirmed. NaG 1:5000, again gave an increase over the heteroauxin control, in this case an increase of 60 per cent. The control, NaG 1:1000 gave a heteroauxin-like curvature, but

TABLE V

EFFECT OF SODIUM GLYCOCHOLATE ON PEA TEST CURVATURES IN HETEROAUXIN

SOLUTION	EFFECT ON PEA STEMS			
	DEGREES CURVATURE (WITH MEAN ERROR) AFTER 12 HOURS	CONDITION	DEGREES CURVATURE (WITH MEAN ERROR) AFTER 24 HOURS	CONDITION
	degrees		*degrees*	
Heteroauxin-H_2O (control)	150° ± 11°	Turgid	173° ± 11°	Turgid
Heteroauxin-Sodium glycocholate, 1: 100,000	164 ± 13	"	224 ± 13	"
Heteroauxin-Sodium glycocholate, 1: 10,000	169 ± 19	"	211 ± 24	"
Heteroauxin-Sodium glycocholate, 1: 5000	269 ± 14	"	290 ± 14	"
Heteroauxin-Sodium glycocholate, 1: 1000	194 ± 15	Flaccid	←(Too flaccid to measure)→	
Heteroauxin-Sodium glycocholate, 1: 500	131 ± 14	"	" " " "	
Sodium glycocholate (control), 1: 5000	− 62 ± 18	Turgid	Negative*	Turgid
Sodium glycocholate (control), 1: 1000	70 ± 17	←(Too flaccid to measure)→		

* Negative curvature signifies no heteroauxin effect.

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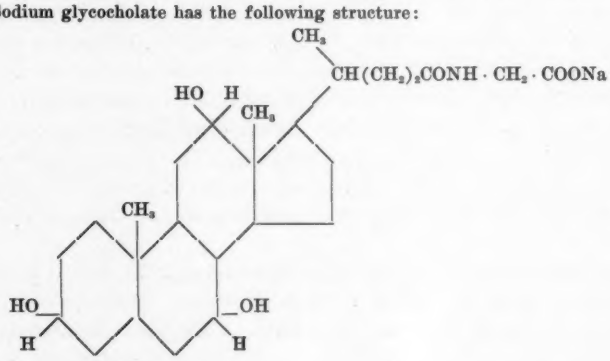
the sections were completely flaccid. NaG 1: 5000 gave no curvature, thus impurities in the NaG do not produce the effect.[4]

It was shown that NaG does not inhibit growth in the pea test. To make the growth test more complete, growth of *Avena* coleoptile sections, infiltrated with NaG and put into heteroauxin solutions with NaG, were measured.

Approximately 4-mm. sections were cut with the VAN DER WEIJ section cutter, placed on teeth of combs, infiltrated with 1: 100,000 NaG, and put in

[4] Sodium glycocholate has the following structure:



0.74 mg. per liter heteroauxin solutions. Twenty-two sections (2 per coleoptile) were used for each test. Measurements were made 12 and 30 hours after the start of the experiment. Table VI presents the results, expressed in millimeters.

TABLE VI

EFFECT OF SODIUM GLYCOCHOLATE ON GROWTH OF COLEOPTILE SECTIONS IN HETEROAUXIN (0.74 MG./L.)

INFILTRATE IN SECTIONS	SECTION LENGTHS				
	0 HOURS	12 HOURS	PERCENTAGE INCREASE IN 12 HOURS	24 HOURS	PERCENTAGE INCREASE IN 24 HOURS
	mm.	*mm.*	%	*mm.*	%
H_2O	4.35 ± 0.01	4.70 ± 0.1	7.9	5.12 ± 0.6	17.7
Sodium glycocholate	4.35 ± 0.01	4.74 ± 0.2	9.0	5.09 ± 0.6	17.0

After 30 hours all sections used in the above experiment showed protoplasmic streaming, and were turgid. It can be concluded that the growth of the *Avena* coleoptile is not affected by NaG, which completely abolishes heteroauxin transport. At first, one might ask how coleoptile growth responds to heteroauxin when transport is abolished? WENT (**44**) has pointed out that polar transport is like ion accumulation in barley roots, potato tuber discs, etc. (**21**), in that the polarity is the result of an excess basal transport over apical transport. Over long periods of time, therefore, polarity will not be limiting, especially in the high concentrations of heteroauxin used. The effect of polarity of transport (and entrance of heteroauxin into the sections) on section growth would probably be observed only in the first few hours, where the velocity of transport of heteroauxin into the control sections would result in a much higher rate than in the sections in which polarity is abolished by NaG treatment. Ordinary diffusion and apical transport ultimately lets enough heteroauxin into the NaG-treated sections to permit growth to overtake the growth of the control sections. Whether or not the above suggestion is correct, the important fact is that growth by cell elongation is unaffected in *Avena* coleoptiles treated with NaG in a concentration which abolishes polar heteroauxin transport.

It can be predicted, *a priori*, that NaG will not inhibit respiration, since growth is unaffected, and since BONNER (**7**) has shown that growth by cell elongation depends on respiration. This prediction was realized by respiration measurements of *Avena* coleoptile sections.

The WARBURG manometric technique (**38**) was employed in making the respiration measurements. Twenty 4-mm. coleoptile sections were placed in

3 cc. of MacIlvane's phosphate buffer (0.005 M) at pH 6 in vessels of approximately 17-cc. volume (vessel constants known). The central wells in the vessels were filled with 0.3 cc. 25 per cent. KOH. The constants were corrected for the volume of the sections. The vessels were shaken at moderate speed in a constant temperature bath at 25° C. in the dark; and readings on the manometers were taken every 30 minutes for 2 hours. One vessel contained sections infiltrated with tap water, another sections infiltrated with 1:100,000 NaG, and the third vessel acted as the barometer. The sections consumed an average of 9.9 mm.^{3} O_2 each 30 minutes in the buffer control; and 9.7 in the NaG-treated. The Q_{O_2} (mm.^{3} per section per hr., at standard temperature and pressure) was 1.21 for the control and 1.22 for the sections treated with NaG. These figures are of the order found by Bonner (7) for *Avena* coleoptile respiration.

The results show that respiration of coleoptile sections is unaffected by NaG of a concentration which abolishes heteroauxin transport. As stated above, this finding would at first sight disagree with Bonner's (7) statement that transport of heteroauxin into coleoptile sections depends upon respiration. Bonner's conclusions were based on experiments in which sections were placed in an O_2-free environment. Van der Weij (40) has also shown that polar transport is reversibly abolished by ether narcosis. The probable explanation is, however, that lack of oxygen and narcosis affect respiratory mechanisms limiting the polarity phenomenon, while sodium glycocholate merely removes the *connecting link* between respiration and transport. To prove that respiration does not limit transport, one would have to abolish respiration without affecting transport, and this has been impossible, so far as the writer is aware. It is conceivable that respiration in certain plant cells which concentrate ions (21) may also be separated from the accumulation mechanism by treatment with similar lysins (this point will be dealt with in a later section of this paper). In other words, respiration may continue without polar transport or ion-accumulation (?), but if respiration is inhibited, both (1) transport (40) and (2) ion accumulation (21) are inhibited.

Thus for the first time, a method has been found which specifically abolishes polar transport. Further experimentation on other physiological processes which are altered during this specific inhibition (*e.g.*, polarization capacity) should reveal the mechanism of polar transport in more detail.

Effect of NaG on lateral heteroauxin transport.—If lateral transport is caused by membrane potentials, as is claimed by many plant hormone workers (see introduction to first paper of this series, 15), it seemed possible that sodium glycocholate would abolish lateral transport of heteroauxin, and therefore photo- and geotropisms. This conception was based on the observation of Ponder and MacLeod (35) that sodium glycocholate and saponin abolished frog skin potentials.

Phototropism.—Avena coleoptiles were removed from etiolated seedlings One-half of the undecapitated coleoptiles were removed from the primary leaves, the other half was left with leaves in place. One-half of each lot was infiltrated with water, the other half with 1:100,000 sodium glycocholate. The coleoptiles were then placed upright, and exposed to a 60-watt gas-filled incandescent lamp (tungsten filament) at 4 meters for 2 hr. at 25° C. (Assuming a mean horizontal candle power of 100 for the 60-watt lamp, the meter-candle-second value becomes approximately 45,000 MCS. The coleoptiles without leaves curve at a faster rate than the ones with leaves, but the final curvature of both sets was the same. Photographs were made of the curvatures, and the measurements and mean error are shown in table VII.

Geotropism.—Coleoptiles prepared as described previously were placed on their sides for 3 hours (25° C. in the dark), after which photographs were taken, and the curvatures measured. Twenty-four coleoptiles were infiltrated with water, and an equal number with 1:100,000 sodium glycocholate. The resulting curvatures are given in table VII.

TABLE VII

EFFECT OF SODIUM GLYCOCHOLATE ON LATERAL TRANSPORT OF HETEROAUXIN IN COLEOPTILES

INFILTRATE	PHOTOTROPISM (24 COLEOPTILES, 45,000 MCS)
	CURVATURE *degrees*
Water	17.8 ± 3.1
Sodium glycocholate, 1: 100,000	20.1 ± 3.3
	GEOTROPISM (24 COLEOPTILES, 3 HR. PRESENTATION)
Water	43.8 ± 5.3
Sodium glycocholate, 1: 100,000	42.7 ± 6.5

It is quite obvious that sodium glycocholate, in a concentration which entirely abolishes longitudinal transport, has no effect on photo- or geotropism. VAN OVERBEEK (**33**) has shown that auxin-a destruction by light may partially account for phototropism in *Avena*, but lateral transport of auxin is the most important factor in photo- as well as geotropism, as has been quantitatively proved by WENT (**41**), DOLK (**17**), BOYSEN-JENSEN (**8**), and others.

It has been stated on a former occasion (**16**) that lateral and longitudinal transport of auxin in plants is caused by entirely different mechanisms. This would be *a priori* predicted, since lateral transport is induced, and may occur toward either side of stems or roots; while longitudinal polar transport

is inherent, and may not be easily reversed, if indeed at all. The difference between the two types of transport was also demonstrated by the facts that gravity, light, and an electric field affect lateral,[5] but not longitudinal, transport.

The difference is further indicated by the unequal effects of sodium glycocholate on the two types of transport. Furthermore, MICHENER (**31**) showed that geotropism may no longer be induced in *Pisum* seedlings after treatment with ethylene.[6]

VAN DER LAAN (**28**) claimed that lateral transport of auxin in horizontally placed *Vicia faba* epicotyls is reversed in ethylene, *i.e.*, that the auxin is displaced to the upper side of the stem. He also showed that ethylene has no effect on longitudinal auxin transport in *Avena* coleoptiles. In general, experiments with ethylene have shown that lateral transport is inhibited or reversed, but that longitudinal transport is unaffected, thus the exact reverse of the sodium glycocholate effect.

The sodium glycocholate effect is not one on electrical polarity, as is shown by the experimental evidence presented in this paper. Evidence from other sources (**15**) has shown a close, if not causal, relationship between lateral displacement of auxin and geoelectric P.D. The irreversible narcosis experiments described in an earlier part of this paper showed that geoelectric P.D. probably constitutes some portion of normal electrical polarity. On such evidence it could be predicted beforehand that sodium glycocholate would not affect lateral displacement of heteroauxin. To what, then, is the effect of sodium glycocholate on longitudinal heteroauxin transport due?

Sodium glycocholate is a lysin, and is highly lipophilic. In high concentration, it probably promotes the solution of lecithin and various lipoids from the cell membranes, and finally completely destroys the membranes. In higher dilutions, however, it may merely compete with the surface-active heteroauxin for surfaces. If heteroauxin is transported either by (1) surface-spreading (**15**), or by (2) adsorption to a larger, cataphoretically transported negative particle, this surface competition would abolish transport. Moreover, as PONDER and MACLEOD (**35**) pointed out, if P.D.'s are abolished by lysins such as sodium glycocholate and saponin, an electrical transport of the heteroauxin anion would be abolished. As yet, no evidence exists which indicates such an electrical transport, but the methods of measurements so far used may be entirely insufficient to demonstrate electrical properties of individual membranes possibly responsible for transport polarity. As pointed out in the section on applied P.D. and unipolar conductance, it is

[5] Lateral transport of auxin in electrotropism has not as yet been directly measured.

[6] Ethylene increases heteroauxin destruction in *Pisum*, but not enough to completely inhibit growth. If growth can occur, geotropism should occur if lateral transport may take place (**31**).

hoped that polarization-capacity experiments will reveal such properties, especially when studied during reversible narcosis. Further, a homologous series of surface-active substance (*e.g.*, the phenyl or indole series of auxins) might show an effect on longitudinal transport of auxin-a or heteroauxin, the effect being greater as higher members of the series are used. This would be interpreted as an increasing competition for surfaces. Polarization-capacity studies made during such treatments might reveal further functional or structural changes in cell membranes due to the action of the surface-active homologues.

Effect of NaG on bromide accumulation by barley roots

This section deals with a few experiments performed in the hope that it might be shown that sodium glycocholate abolished ion accumulation in barley roots, a phenomenon shown by Hoagland and Broyer (**21**) to be dependent upon oxidations, just as is polar transport. Furthermore, Went (**44**) indicated a similarity between the two, and the present paper has already suggested similarities. It will be shown, however, that there is no effect of sodium glycocholate on bromide accumulation in roots.

Barley seeds were soaked in tap water 24 hours, planted in chloride-free sand on wire mesh, germinated in tap water in the dark room at 25° C. and 90 per cent. relative humidity until about a week old, after which they were transferred into the diffuse light of the laboratory for 2 hours, and then into the green house. Hoagland and Broyer (**21**) substituted chloride-poor solution for the tap water. After the root system was about 2 weeks old, the roots were excised, washed with distilled water twice, weighed, dried at 100° C. for an hour, and reweighed. The difference between wet and dry weights was assumed to be proportional to the sap content. The dried roots were digested in Na_2O (0.5 gm. per gm. wet weight), the digest evaporated on a hot plate, neutralized to congo-red paper, filtered, the residue washed several times with hot water, and the collected filtrates titrated electrometrically with a silver bromide electrode (against a calomel electrode, using a KNO_3-agar bridge) for total bromide (**20**). Excised roots were placed in flasks containing chloride-free and salt-poor solutions (**21**) containing sodium glycocholate, 1:100,000, 1:10,000, and 1:5000, and the controls containing no sodium glycocholate. Another lot was run in which this setup was duplicated, but in addition to which NaBr (0.005 M) was added. The flasks were vigorously aerated by bubbling oxygen through sintered glass. After 12 to 24 or 48 hours, the roots were removed, washed twice in distilled water, and analyzed for bromide. No chlorides were present in the controls.

The electrometric endpoints were highly satisfactory, and easily obtained. Sodium glycocholate had absolutely no effect on bromide accumulation by these roots. Table VIII shows two typical cases.

TABLE VIII

EFFECT OF SODIUM GLYCOCHOLATE ON BROMIDE ACCUMULATION BY BARLEY ROOTS

EXPERIMENT	MILLIEQUIVALENTS Br^- PER LITER			
	A NaBr IN SAP	B NaBr IN SOLUTION	C AMOUNT ACCUMULATED (A–Ac*)–B	D ACCUMULATION RATIO (A–Ac*)/B
6/17/37 Accumulation time (12 hr.)				
Control	0.541 (Cl^-?)	0	.....	.....
NaBr	11.82	4.75	6.53	2.38
NaBr + Sodium glycocholate (1: 10^5)	13.20	4.75	7.91	2.66
6/21/37 Accumulation time (15 hr.)				
Control	0.114 (Cl^-?)	0	.....	.....
NaBr	15.30	5.00	10.20	3.04
NaBr + Sodium glycocholate (1: 10^4)	16.9	5.00	11.80	3.36

* Ac = A control.

A few experiments on bromide accumulation in *Nitella* showed that 1:100,000 sodium glycocholate was toxic, and allowed electrolytes already present in the sap to escape.

It must be concluded that ion accumulation in roots, and polar transport of auxins are not brought about by the same types of mechanisms. It was therefore conjectured that only surface-active ions will display polar transport, or be prevented from accumulating. A few experiments were then performed in which it was attempted to demonstrate accumulation of heteroauxin by *Nitella* cells, using the $FeCl_2$ colorimetric test for heteroauxin ($FeCl_2$ and concentrated H_2SO_4 are boiled with heteroauxin. 1:10^6 will give a pink color) in expressed sap of cells placed in heteroauxin solutions (tap water) from 1 to 50 mg. per liter, which gave very clear tests by this colorimetric method, both before and after the experiments. The test was, however, much weaker after a run than before, and no test was obtained inside the cells (expressed sap). It was concluded that either (1) heteroauxin is completely destroyed in the sap, once it penetrates, or (2) that it is present in concentrations too small to be detected by this method, and therefore is not accumulated. The cells were probably quite healthy and normal, as shown by the fact that they contained nearly 0.1 N in chlorides

(as tested by electrometric titration with a chloride electrode), while unhealthy cells frequently lose their accumulated ions (**21**).

The writer suggests further experiments on the effect of lysins on accumulation of organic and inorganic electrolytes and nonelectrolytes, including surface-active ones.

Summary

1. Sections of *Pisum* stems and of *Avena* coleoptiles are reversibly polarized by applied direct currents beyond certain thresholds of applied current. The normal electrical polarity may be reversibly increased or decreased, or reversed by these currents.

2. Apical negativity (normal polarity) is increased, within limits, on stimulation by alternating currents, but only after certain thresholds of applied currents are reached.

3. *Avena* coleoptiles cannot act as ohmic rectifiers of an alternating current, as claimed by METZNER (**30**) for many plant tissues, nor do they display a unipolar resistance to a direct current. This means that a possible asymmetry in membrane permeability theoretically linked with polar transport (accumulation, secretion?) cannot be analyzed by simple unipolar or rectification measurements.

4. The apical negativity of *Avena* coleoptile sections is reversibly decreased by ether narcosis, and the P.D. drops to that caused by gravity when narcosis is irreversible.

5. The resistance of *Avena* coleoptiles reversibly decreases on passage of small direct currents and alternating currents.

6. The resistance changes follow the inherent P.D. changes, but in the opposite sense, *i.e.*, when apical negativity increases, the ohmic resistance decreases. An exception occurs in complete irreversible ether narcosis, where both resistance and P.D. decrease.

7. Polar heteroauxin transport in *Avena* coleoptiles may be specifically abolished with 1 part of sodium glycocholate in 100,000 parts of water, without there being any change in *electrical polarity*, respiration, semi-permeability, growth by cell elongation, or protoplasmic streaming.

8. Lateral and longitudinal transport of auxin-a in plants are caused by entirely different mechanisms, as shown by the different effects of light, gravity, applied potential differences, ethylene (**31**), and sodium glycocholate.

9. The apparent relation of auxin transport in plants to ion accumulation by barley roots, as suggested by WENT (**44**), is shown to be somewhat questionable, as sodium glycocholate abolishes the former but has no effect on the latter.

10. Statements 1 to 7 lend additional support to former statements made by the writer (**15, 16**) that electrical polarity, expressed in terms of

inherent potential differences (unipolar conductance, or rectification of alternating current) has no apparent causal relation to polar auxin transport in plants.

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CONDUCTIVITY MEASUREMENTS OF PLANT SAP[1]

GLENN A. GREATHOUSE
(WITH TWO FIGURES)

Introduction

The plant physiologist recognizes more and more the importance of the application of physics and physical chemistry to fundamental plant problems, realizing that a mastery of botany, physics, chemistry, and mathematics will aid in the understanding of the living cell.

Conductivity measurements have been widely used in the study of plant problems in recent years (**7, 8, 12, 14, 15, 17, 25, 27, 28, 32**). A critical examination of these articles indicates the desirability of reviewing the basic principles of conductivity measurements and of pointing out the various factors that influence the conductivity value of plant sap. This paper will discuss the application and limitation of the measurement of electrical conductivity of plant saps, with particular reference to cold-hardened and unhardened plant tissues.

Experimental determination of conductivity

The conductivity of an electrolyte is measured in terms of the resistance of the solution between two electrodes, and it is the reciprocal of the resistance. The resistance may be measured by the alternating current Wheatstone bridge method as employed by Kohlrausch or by modification of this method. For the theory of bridge measurements the reader is referred to standard textbooks of physics and physical chemistry.

The essential features of an apparatus for measuring the resistance of an electrolyte by the Kohlrausch method are indicated in figure 1. In this diagram C is the electrolytic cell, R is a variable resistance, X is the condenser, E is a source of alternating current, T is the current detector, a and b the ends of the bridge, and d the movable contact. R_1 is a resistance coil (the resistance of each coil is usually $4\frac{1}{2}$ times that of the wire, making the latter 0.1 of the total resistance of the ratio arms) and K is a switch to short circuit the coil R_1.

Kohlrausch's classical method has had the benefit of numerous improvements in recent years. The researches of WASHBURN (**38, 40, 41**) have been

[1] Measurements of electrical conductivity have many applications in physiological investigations. The importance of knowing the underlying principles involved in such measurements as well as the limitations of application to plant physiological problems cannot be overemphasized. In this report prepared by Dr. GLENN A. GREATHOUSE for the Physical Methods Committee measurements of conductivity and their applications are discussed. This report, together with its bibliography, should be helpful to those working in this field of research.—*Earl S. Johnston, Chairman.*

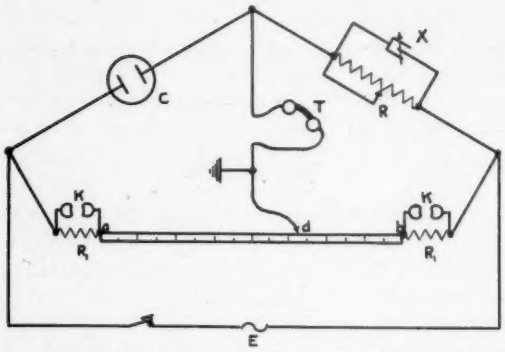

FIG. 1. Diagram of Wheatstone bridge for measurement of electrolytic resistance.

particularly valuable in stimulating accurate work in conductivity measurements. Of particular importance from the standpoint of precision measurements has been the introduction of vacuum tube alternating current generators and amplifiers for providing currents of symmetrical wave form, on the one hand, and increasing the sensitivity of the detector on the other (**11, 19, 20**). JONES and JOSEPHS (**20**) have described in a detailed paper a direct reading alternating current bridge embodying these new features. Their paper also includes a valuable and comprehensive study of various sources of error from the electrical standpoint in the Kohlrausch method as it has been generally used. STONE (**35**) has likewise described a vacuum tube potentiometer for the measurements of the conductivity of solutions. The circuit employs two thermionic tubes, the plate currents of which are visually balanced by a very sensitive null-point galvanometer through the adjustment of grid potentials derived from small batteries, and the superimposed alternating current used in the measurement.

For wiring diagrams of modifications and improvements over the classical Kohlrausch method the reader is referred to articles by HALL and ADAMS (**11**), SHEDLOVSKY (**33**), and STONE (**35**).

Factors affecting accuracy of conductivity measurements

In measuring the conductivity of solutions it is desirable to use a low voltage in the bridge. This may mean a modification of the oscillator to give low and controllable voltages. The use of low voltages in the bridge diminishes its sensitiveness, and, as a result, the amplification must be improved. The principles of design of a transformer for a vacuum tube amplifier are described by JONES and BOLLINGER (**19**) and STONE (**35**).

The resistances used must be as free as possible from the effects of inductance and capacitance at the frequency of the current in the bridge

circuit; also, attention must be given to protecting the bridge against external influence caused by capacitance and inductance. JONES and BOLLINGER (19) state that the mutual inductance between the oscillator and the detector, and between the oscillator and the bridge, may cause an error in the measurement of the conductivity of solutions when low voltages are being used. They give means for the discovery and elimination of this error.

For precision work, the electrostatic screening of the bridge is essential. An unscreened bridge is affected by the movements of the observer. Without adequate shielding, delicate balances are difficult to obtain because of shifting capacities introduced by the hand of the operator, etc. Also, only with proper screening are measurements of high precision possible under conditions existing in modern laboratories in which other electrical work may be in progress.

The experimenter must determine the desired accuracy needed in the oscillator, the current detector, etc. References on these points can be found in papers by SHEDLOVSKY (33) and WASHBURN and PARKER (41).

Balancing of the circuit depends upon instantaneous equality of potential at the terminals. This condition cannot be established if the currents in the two parts of the bridge are out of phase. If the conductivity of the electrolyte is high the current flowing in the cell may be out of phase with that of the resistance on account of the electrostatic capacitance of the conductivity cell. Such a condition prevents perfect balancing of the system. In precise measurements an adjustable condenser is connected across the terminals of the balancing resistance to annul the capacity of the cell.

The use of properly constructed conductivity cells and platinized electrodes is essential. The use of cells of high cell constant (length divided by cross section) is recommended so that the resistance to be measured will be high (10,000 to 30,000 ohms), thereby reducing polarization and heat effects in the cell. A convenient test of the quality of cells is observation of the variation in apparent resistance with variation in frequency. Cells with sliding electrodes, or electrodes easily distorted, should be avoided. The reader is referred to a paper by WASHBURN (38) for different types and sizes of conductivity cells adapted to measurement of solutions possessing low to high conductivity. He gives formulae to calculate the area of electrodes and the distances between these electrodes to be used for different ranges of conductivities, together with the minimum volume for each type of cell.

In most cases the platinum electrodes should be coated with platinum black, which increases the surface area and aids in reducing the surface density of ions deposited, to which the E. M. F. of polarization is proportional. The platinum electrodes must be thoroughly cleaned before being

coated with platinum black. This may be done by pouring enough concentrated HCl into the cell to cover the electrodes. The electrodes are connected with a current of 1.5 to 6 volts through a reversing switch and then electrolyzed by reversing the current every 30 seconds until all of the old platinum black is stripped off. The hydrochloric acid is then poured out and the cell rinsed. Now the electrodes are covered with platinizing solution and electrolyzed with 30-second reversals for 10 minutes or more. A resistance is often inserted in the system so that a gentle evolution of gas occurs, and a fine deep black coating of platinum, free from lead, is deposited. The platinizing solution consists of 1 gm. of chloroplatinic acid and 0.008 gm. of lead acetate in 30 ml. of water. After the electrodes are well coated with platinum black, the cell is immersed for a number of hours in warm distilled water, frequently changed to remove absorbed salts. Finally, 6 N H_2SO_4 is poured into the cell which is connected with the source of the current and electrolyzed with 30-second reversals for a period of about 15 minutes. This treatment removes from the electrodes the last traces of platinizing solution and occluded chlorine. The electrodes are then rinsed and stored in distilled water. They should not be allowed to dry out.

Conductivity cells for low conductance solutions should be lightly platinized and those for high conductivity solutions should be heavily platinized. The cells should be carefully annealed and aged before use. Likewise, large temperature changes should be guarded against as they lead to thermal strains which may alter the distance between electrodes.

The cell constant applies only to a particular temperature. Kohlrausch has recommended several solutions of known conductivity for standardization of the electrolytic cell, but in many cases these are suitable only for approximate work. His standard is normal KCl solution made up from 74.555 gm. of pure KCl weighed in air and diluted to one liter at 18° C. This solution should have a conductivity of 0.09822 and a density of 1.04492 at 18°. Frequently the normal solution of KCl is diluted to 0.02 N and used for determining the cell constant. This solution at 18° has a conductivity of 0.002397 according to Kohlrausch.

PARKER and PARKER (29) recommend that, with the ordinary definition of cell constant, the concentration of the solution should be expressed in equivalents per cubic decimeter, not per liter, and unit concentration is then demal, not molal or normal. They also find that the value of the cell constant depends on the voltage and frequency of the current, and on the electrolyte used. They suggest that the standard solution be made from 76.6276 gm. of pure KCl weighed in air and added to 1000 gm. of water also weighed in air. The standard solution may then be diluted as in solutions recommended by Kohlrausch to 0.1 to 0.01 N. The conductivity of the

one demal solution was found by PARKER and PARKER (**29**) to be 0.097790 at 18°. This value is lower than that given by Kohlrausch. Tables of values for temperatures from 0° to 30° are given along with a comparison of previous data.

WASHBURN (**38**) determined the cell constant with a solution of 7.4300 gm. KCl in 1000 gm. of solution (weights in air). This solution produced a conductivity of 0.01288 at 25°, in contrast with that of PARKER and PARKER, of 0.0128524 at 25°. It is good practice to ascertain the known conductivity of a given solution as recorded in the International Critical Tables.[2] The writer's experience has shown that these tables as given in textbooks may contain errors.

It is necessary to have a balance between the resistance of the lead wires to the conductivity cell and that of the wire connecting the resistance box with the bridge. Otherwise the lead resistance must be determined by measuring the resistance of the cell when filled with mercury and this resistance subtracted from the measured resistances.

Alternating current of high frequency is necessary to prevent the polarization of the electrodes which occurs when direct current is used (**36, 38**). Currents of 1000 cycles or more should be used to minimize polarization. Polarization can be minimized and made practically insignificant by a combined use of proper platinization, large electrodes, high frequency, and high resistance. Experiments have shown that, if polarization is eliminated, Ohm's law can be accurately followed, and the actual values of the applied potential and current need not be known as they cancel out in the final calculation of conductivity.

Since the resistance of an electrolyte is affected appreciably by temperature, the conductivity cell must be immersed in a bath, the temperature of which is kept constant and measured accurately. Water may be used as a bath liquid, but in some cases in which the conductivity of the electrolyte is low, an oil bath is preferable. A temperature difference of 1° C. changes the value of the conductivity of the solution about 2 per cent. In order to obtain an accuracy to 0.1 per cent., temperature control of 0.05° is necessary.

Good insulation between the various parts of the apparatus is essential.

Conductivity measurements in pure and biological solutions

The conductivity of pure liquids is small. Purest water obtained by KOHLRAUSCH and HEYDWEILLER (**23**) had a conductivity of 0.043×10^{-6} at

[2] JONES, G., and PRENDERGAST, M. J. Jour. Amer. Chem. Soc. 59: 731–736. 1937: "the absolute specific conductance of the 1, 0.1 and 0.01 N KCl solutions recommended by Kohlrausch as a standard of reference for conductivity measurements have been redetermined. The results indicate that the corrections used by the 'International Critical Tables' in the recalculation of the conductance data are unreliable."

18°. If pure water is allowed to reach equilibrium with the CO_2 of the air, its conductivity increases and is about 0.8 to 1×10^{-6}.

The interpretation of conductivity values obtained from single component solutions, pure and dilute, is much simpler than those secured from mixed or biological solutions. The conductivity values obtained from plant saps[3] must be interpreted cautiously. It is almost impossible to translate conductivity values of mixed salts or plant saps into concentration values. Accordingly, one cannot calculate from electrical conductivity measurements the concentration of salts in plant saps. DEXTER (**3**) and others have attempted to translate conductivity values into salt concentrations. DEXTER states that "the salt per gram of water in the plant decreased as the season advanced, in spite of the fact that the amount of water decreased. That is, the concentration of salt in the sap, assuming all water to be free for solvent purpose, decreased regularly." HARRIS, GORTNER, and LAWRENCE (**12**) have suggested the use of the ratio $\frac{k}{\Delta}$, as of value in indicating changes in the ratio of electrolytes to non-electrolytes in plant saps. PASCOE (**30**) found that the depression of the freezing point in the mixture of $MgCl_2$ and $MgSO_4$ is a linear function of the mixture, whereas the conductivity curves is not a linear function in mixed solutions. PASCOE's results, as well as the writer's (**7, 8**), indicate that the conclusion of DEXTER and the use of a ratio by HARRIS, GORTNER, and LAWRENCE are unwarranted.

Factors that may affect the conductivity of plant saps are: (1) hydration of colloids and crystalloids (frequently recorded as "bound water" or "unfreezable water"); (2) viscosity; (3) adsorption of ions by colloids (colloids differ in their ability to adsorb certain ions (**16**); (4) nonelectrolytes; (5) change in the dielectric constant of the system, as a result of the action of colloids, organic acids, etc.; (6) surface conductance of colloids; (7) concentration and solubility of the crystalloids; (8) temperature; and (9) size of molecule of crystalloids.

WIEDEMANN (**42**) was the first to point out a connection between conductivity and viscosity. From his work on solutions of $CuSO_4$, he formulated a relation $\frac{ky}{p} = \text{constant}$, where k is the conductivity of a solution of concentration p, and y is its viscosity. The resistance offered to the motion of an ion in solution because of the viscosity may be expected to have some influence on conductance. For single component systems of higher concentrations, the calculated ionization has been shown to change by 8 per cent. depending upon whether or not viscosity is taken into account. It is quite evident that viscosity influences the conductance of plant saps (tables I, III).

[3] For a discussion of methods of extracting of plant saps see Plant Physiol. **4**: 103. 1929; **7**: 439. 1932.

GREEN (**10**) working with sugar, salt, and acid solutions, showed that conductivity did not vary directly as fluidity, but to some function approximately $f^{2/3}$. The power 0.55 was found to give the best results for the experiments with HCl, and 0.70 when KCl was the electrolyte used.

Table I presents representative data that indicate the influence of some of the factors involved in the interpretation of conductivity measurements on plant tissues. The red clover plants used in this study were a portion of the samples collected on January 30, 1935, for which a chemical analysis has been published (**8**). The cabbage plants were of the Early Jersey Wakefield variety. These plants were grown until 67 days of age under identical cultural and environmental conditions. At the end of this period the plants were divided into two groups. One group of plants was left in the greenhouse while the other group was placed in the hardening room at 40° F., remaining there for an additional period of 12 days. The plants were illuminated by means of 100-watt Mazda lamps placed at a distance of 3 ft. from the plants. During the growth of these plants, an attempt was made to control all variables except the temperature at which the plants were grown for the 12-day period previous to sampling. The *Bryophyllum* plants were grown in the general plant physiology greenhouse. The methods used in these analyses were similar to those previously discussed (**7, 8**).

TABLE I

PHYSICAL AND CHEMICAL MEASUREMENTS OF PLANT TISSUES

TISSUE	TOTAL MOISTURE	UNFREEZABLE WATER −22° C. % TOTAL WATER	SPECIFIC CONDUCTANCE $\times 10^{-3}$ 25 ± 0.02° C.	VISCOSITY (RELATIVE)	pH	TOTAL SUGAR % DRY WT.	PROTEIN NITROGEN* % DRY WT.	NONPROTEIN NITROGEN* % DRY WT.
	%	%				%	%	%
Cold-hardened red clover roots								
French variety	80.60	17.37	1069	1.146	5.24	10.86	1.527	1.278
Ohio variety	77.90	20.46	865	1.250	5.57	13.40	1.526	1.326
Cabbage (shoot) 79 days old								
Unhardened	90.32	8.30	1637	1.356	5.26	5.68	1.617	0.532
Hardened	88.32	9.53	1331	1.407	5.32	8.23	1.376	0.766
Bryophyllum								
Leaf blade	93.79	1.59	734	1.136	4.03	.....	.....	.....
Young plants from leaf blade	83.25	4.18	616	1.251	4.49	.....	.....	.....
Leaves from three lower nodes	93.60	1.76	741	1.146	4.12	.....	.....	.....
Leaves from three upper nodes	91.73	3.25	625	1.198	4.83	.....	.....	.....

* The nitrogen determinations were made by DR. NEIL W. STUART.

In the interpretation of the data of table I it is essential to keep in mind that the carbohydrate, nitrogen, total water, and unfreezable water analyses were made on the whole tissue, while the conductivity, viscosity, and pH determinations were made on the plant sap. The data of table I show that with each plant type the conductivity varied inversely with the relative viscosity, the unfreezable water, and the total sugar content. These data show that the total water content is positively correlated with the conductivity, while the unfreezable water and nonprotein nitrogen values are negatively correlated with conductivity. No consistent relationship between protein nitrogen and conductivity was found. It has been previously pointed out that there was no consistent relationship between total water content of roots and shoots and the hydration measurements (**9**). Neither was the protein nor the nonprotein nitrogen found to influence markedly the hydration capacity of the tissues.

KRUYT (**24**) states that investigations made in his laboratory have forced him to assume that hydration, which is without doubt closely connected with the orientation of the water dipoles, must be understood as meaning that the dipoles which are situated in the neighborhood of a particle are in complete orientation and that those somewhat more remote are less perfectly orientated. If this is the case, the degree of hydration would greatly influence the solubility of the salts present, and the tissue with the greater degree of hydration would have the lower conductivity.

It is difficult to distinguish cause from effect in conductivity measurements of plant sap. Nevertheless, with a critical examination and application of both physical and chemical methods of analysis to plant tissues the relation between cause and effect of conductivity of plant saps should become clearer.

The same basic factors may be responsible for lower conductivity in cold-hardened tissue and in tissue of different age and position on the same plant (table I). Other investigators, when studying the gradients in plants, have noted a lower conductivity with younger tissue. This shows how difficult it is to interpret conductivity measurements even within the same plant. From the data in tables I and II and from those of other investigators, evidence points to the possibility that ions bound to the colloidal phase could offer considerable protection to loss of water from plant tissues.

In order to determine whether this lower conductivity was due to lower ion or salt content, sap was expressed from plant tissues, the conductivity determined, and 50 ml. of this plant sap brought to volume in an accurate thermostat. This volume of plant sap was electrodialyzed until free of all removable ions and again brought back to the original volume and the conductivity redetermined. The results of this experiment are presented in table II.

TABLE II

CONDUCTIVITY MEASUREMENTS ON PLANT SAP BEFORE AND AFTER ELECTRODIALYSIS

PLANT SAP FROM	CONDUCTIVITY OF SAP BEFORE ELECTRODIALYSIS $\times 10^{-6}$ 25 ± .02° C.	CONDUCTIVITY OF SAP AFTER ELECTRODIALYSIS $\times 10^{-6}$ 25 ± .02° C.	DIFFERENCE
Cold-hardened red clover roots			
French variety	1069	1106	37
Ohio variety	865	1023	158
Cabbage plants 79 days old			
Unhardened	1637	1698	61
Hardened	1331	1601	270
Unhardened red clover roots			
French variety	873	895	22
Ohio variety	708	726	18

These data indicate that the differences in conductivity in these tissues are not caused entirely by potential ion content, but that some of the ions are probably adsorbed, or that ionization of the salts is influenced by viscosity, dielectric changes, etc.

The influence of non-electrolytes on conductivity of salt solutions has been known for many years. Very few, if any, measurements have been made of the influence of nonelectrolytes on plant saps. Table III gives data to show the effect of sucrose on the conductivity of plant saps.

TABLE III

EFFECT OF SUCROSE ON CONDUCTIVITY OF PLANT SAP

SUCROSE ADDED TO PLANT SAP	CONDUCTIVITY OF PLANT SAP $\times 10^{-6}$ 25 ± .02° C.		DIFFERENCE
	ORIGINAL	AFTER ADDITION OF SUCROSE	
Normality			
0.02	805	788	17
0.1	805	745	60
0.2	805	701	104
0.4	805	724	81

It can be noted from table III that the conductivity of the plant sap decreases on the addition of sucrose in small amount, probably because of decrease in ionization and the mobility of ions as a result of change in viscosity. With further addition of sucrose the conductivity increased, possibly because of hydration and concentration effects on the nonelectrolytes. The addition of other sugars, such as glucose and fructose, to plant saps gave results similar to those of the experiments with sucrose differing, however, in the concentration needed to produce a decrease in conductivity. The results in table III were corrected for the conductivity of the sugar solutions.

The conductivity and the freezing point depression of solutions of sucrose in distilled water were determined in order to note the influence of different concentrations. These results are presented graphically in figure 2.

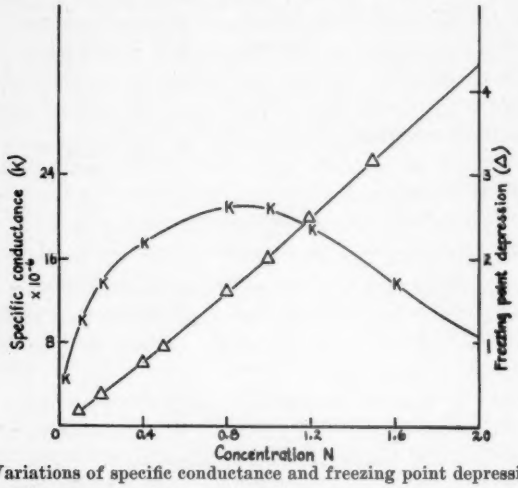

FIG. 2. Variations of specific conductance and freezing point depression with change in concentration of sucrose.

The conductivities shown by these sugar solutions are probably caused, not only by small quantities of impurities present, but also by a slight ionization of the sugar molecule itself, since other workers have shown that sucrose is to be regarded as an extremely weak acid. The decrease in conductivity after the solution has reached a concentration of 0.8 N is probably the result of a decrease of ionization and mobility of the ions.

The depression of the freezing point curve for sucrose deviates progressively from a straight line in a manner indicative of a greater apparent degree of hydration of the sucrose molecule at higher concentrations. In other words, the sucrose does not attract the (HOH_n) molecules to an extent always equivalent to the formation of a hexahydrate, as suggested by SCATCHARD (**31**), but increases in its apparent degree of hydration with decrease in the relative activity of the water in the solution. This may have some bearing on the conductivity values of plant saps.

As it was thought possible that inversion of the sucrose might be brought about by the presence of materials in the plant sap, with a consequent small alteration of the viscosity, a solution was kept for an hour and examined by polariscope and reduction, but little inversion could be detected, and the effect of such a source of error may be dismissed as negligible.

There are a number of other possible factors that might be responsible

for deviations in conductivities of plant saps. MARINESCO (**26**) has found that the dielectric constant is lowered by the addition of colloids to the solvent water.[4] On the basis of these findings it is probable that the dielectric constant is lowered by water-binding of the cell constituents. Therefore, the tissue with the greater water-binding capacity should have less dissociation of electrolytes and consequently a lower conductivity. A number of investigators have noted a change in organic acids with change of the environment of the plants. These changes in acid content would likely influence the hydration of the system and its dielectric value.

There is always the possibility that a certain portion of the conductivity in plant saps is caused by the surface conductance of colloids. BRIGGS (**2**) found that the electrical conductivity of a colloid gel was not necessarily related to the presence or the concentration of ionized electrolytes. He noted that a membrane of pure cellulose immersed in conductivity water conducted a current, and he was able to demonstrate that the conductivity was not due to inorganic constituents present in the system. In a more recent paper, BRIGGS has demonstrated that there is a definite lyotropic series of ions which influence surface conductance. As a result of these investigations there is indication that the conductivity of plant saps is in part associated with surface conductance through the lyophilic colloidal gel structure, as well as with ionic conductance of the solvent.

Application of conductivity measurements

Pure water is essential for most laboratory work and it is necessary to know from time to time the conductivity of the distilled water used as a solvent. These data can be obtained quickly and accurately by means of the conductivity apparatus. Distilled water for general laboratory purposes should not have a conductivity greater than from 1.5 to 2×10^{-6} and for work of greater precision water of conductivity 1×10^{-6} or better should be obtained. In precision conductivity measurements there is a water correction. This correction has been discussed by WASHBURN (**39**) and by KENDALL (**21, 22**).

The principle of increased permeability and electrical conductivity of injured tissue is a familiar one to the plant physiologist. The application of this method to different plant problems can be found in papers by HEALD (**14**), HIBBARD and MILLER (**15**), HOAGLAND and DAVIS (**17**), HOTTES (**18**), MERRILL (**27**), and OSTERHOUT (**28**).

The diffusion of electrolytes from tissue exposed to low temperature in distilled water has been determined electrometrically by DEXTER and asso-

[4] In this connection it is interesting to note that ALEXANDER and SHAW (1) have recently devised a method for the determination of ice-water relationships by measurements of dielectric constant changes.

ciates (**4**). The magnitude of the electrical conductivity has been associated with the known hardiness of the tissue. GREATHOUSE and STUART (**7, 8**) have applied conductivity measurements of plant sap to the problem of cold hardiness. According to the work of DEXTER, and of GREATHOUSE and STUART, the degree of cold hardiness of plants is related to the ions that move by diffusion from the tissue or to the ions free in the plant sap. This measurement, however, does not give the true ionic relationship in these tissues (tables II, III).

The production of CO_2 in respiration has been measured electrometrically (**13, 34**).

Conductivity measurements are very useful in determining when the substrata are free of their ions after electrodialysis of plant materials.

The physical chemist has used this measurement for the determination of (a) the solubility of difficultly soluble salts; (b) the basicity of organic acids; (c) the degree of hydrolysis; (d) the end-points in titration reactions; and (e) the dissociation of water and other solutions.

Precautions in electrical conductivity measurements

If the proper apparatus is assembled, connected, and adjusted correctly it is possible to make conductivity measurements with the great degree of precision which is necessary in all research laboratories in plant physiology. The application of this measurement to some of our plant problems has proved of great importance. The method has practical value even though its results cannot be interpreted in terms of ion concentration and behavior. If the true ion concentration and behavior are required, it will be necessary to determine these properties through additional experimentation.

It is obvious that anyone who is to make conductivity measurements should know the basic principles of the apparatus as well as those of the ionic relationships of solutions. One should know how to design and platinize electrolytic cells for solutions of low or high conductivities. Other factors of equal importance have been previously mentioned in this paper.

Errors in the conductivity measurements must be guarded against if it is to be a useful tool to plant science. Errors produced by polarization at the electrodes, changes in temperature, etc., on conductance are or should be common knowledge. It is the less obvious errors that are frequently ignored.

Electrical units and laws pertaining to conductivity

Ohm, in the well known law ($E = RIk$), summarized the relation between resistance, current, and pressure, where R is the resistance, E the electric pressure, and I the current, while k is a factor depending only on the choice of units and can be made equal to unity.

The unit of electrical resistance is the International Ohm and is defined as the resistance offered to an unvarying current by a column of mercury at the temperature of 0° C., 14.4521 gm. in mass, 106.3 cm. in length, and of constant cross section.

The unit of current is the International Ampere and is defined as the unvarying electric current which when passed through a solution of silver nitrate in water, in accordance with specified conditions, deposits silver at the rate of 0.001118 gm. per second.

The unit of electric pressure is the International Volt and is defined by Ohm's law as the electric pressure which, when steadily applied to a conductor the resistance of which is one International Ohm, will produce a current of one International Ampere.

Both the ampere and the volt are based upon the fundamental law of Faraday, which shows the relation between the current and the amount of chemical reaction that takes place when a current passes from a metallic conductor to an electrolytic conductor. Faraday's law is divided into two parts and may be expressed by the following relationships: (1) the amount of any substance deposited by the current is proportional to the quantity of electricity flowing through the electrolyte, (2) the amounts of different substances deposited by the same quantity of electricity are proportional to their chemical equivalent weights.

The specific resistance, or resistivity, of an electrolyte may be defined as the resistance in ohms of a centimeter cube of solution. Specific conductance, or conductivity, is the reciprocal of specific resistance.

The molar conductance is the conductivity, in reciprocal ohms, of a solution containing one mol of solute when placed between sufficiently large electrodes which are exactly 1 cm. apart. The equivalent conductance may be defined in the same terms as molar conductance, if one gram-equivalent of solute is substituted for one mol of solute.

It is obvious that the construction of an electrolytic cell with electrodes suspended exactly 1 cm. apart and each electrode having 1 sq. cm. of area, would be difficult. To avoid this difficulty a correction is made and is incorporated in the value known as the cell constant.

The cell constant, C, is defined by the equation $k = C/R$ in which k is the specific conductance of a solution and R the resistance of the cell containing that solution. The cell constant may be calculated from a measurement of R by the usual procedure when a solution (usually 0.1 or 0.01 N KCl) of known k is in the cell.

Summary and conclusions

1. The basic principles, the apparatus, and the precautions needed in determining the specific conductance of solutions are discussed.

2. Data are presented which indicate that conductivity values obtained from plant sap must be interpreted cautiously.

3. A list of possible factors that may affect the conductivity values of plant sap is presented and each factor discussed.

4. The electrodialysis data indicate that the lower conductivity of the expressed sap from cold-hardened plants was not due entirely to fewer ions, but was probably influenced by adsorption, viscosity, ionization, etc.

5. The conductivity of plant sap was found to decrease on the addition of nonelectrolytes in small amounts. With further addition of nonelectrolytes the conductivity increased.

6. The interaction of the solute and solvent is of major importance in the interpretation of electrical conductivity data.

7. The application and limitations of specific conductance measurements are given.

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BEHAVIOR OF PECTIC SUBSTANCES AND NARINGIN IN GRAPEFRUIT IN THE FIELD AND IN STORAGE

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(WITH FIVE FIGURES)

Introduction

The physiological breakdown of citrus fruits variously known as storage spot, pitting, or pox has presented a perplexing problem to growers and shippers ever since the storage and transportation of these fruits was undertaken on an extensive scale. This breakdown manifests itself in various forms of blemishes of the rind, depending on such factors as the inherent condition of the fruit, the temperature at which the fruit is held, the length of storage, and the composition of the atmosphere surrounding the fruit. The disease is associated with exposure to excessively low temperatures, but its development is conditioned by the other factors mentioned. Partial control of this disorder has been attained in commerce by raising the temperature at which the fruit is held during transit and storage, the temperature depending on the kind of fruit in question. California oranges hold well at 38° F., but California-grown lemons and grapefruit require a temperature of 50° to 55°, and recommendations of temperatures as high as 65° have been made for commercial grapefruit storage. Other factors enter into the consideration of the best holding temperature; *e.g.*, grapefruit will develop a deeper yellow or bronze at a temperature of 55° than at 65°. This deep yellow color is considered undesirable by the trade because of the appearance but has no ill effect on the quality of the fruit. On the other hand, fruit held at 65° will be more subject to attacks by the various organisms of decay. High relative humidities are necessary at these higher temperatures in order to reduce shrinkage, but also afford favorable conditions for the development of these organisms.

It has been shown previously (**6**) that losses from pitting in grapefruit can be reduced by choosing the proper season for picking fruit which is to be held or which is to be shipped great distances under refrigeration. Additional evidence on the influence of season on the susceptibility of grapefruit to pitting will be submitted in this paper. The nature of the changes occurring in the fruit during its development on the tree which tend to increase or decrease its susceptibility to pitting if subjected to the unfavorable environment of low storage temperatures is not known. Evidence has been presented previously (**6**) and additional evidence is presented in this paper indicating that the temperature in the grove during the period immediately preceding picking has a bearing on the susceptibility the fruit will have to

pitting upon subsequent storage. Evidence is also presented that certain concomitant changes take place in the composition of the rind of the fruit. These changes may have a partial responsibility in bringing about variations in susceptibility to pitting, but other changes which have not been noted may play a more important part.

Material and methods

Although the general problem of the pitting of the rind arises in several kinds of citrus fruits, this report will be limited to work on the Marsh variety of grapefruit (*Citrus grandis* (L.) Osbeck). In order to obtain information on the possible effect of the place of production on the holding quality of the fruit, collections were made from three widely separated groves: (1) Near Oasis, California, in the Coachella Valley; (2) Corona, in the western part of southern California; and (3) Woodlake, in the central California citrus district. At Oasis the collecting period extended from October 29, 1935, to April 14, 1936; at Corona from March 10, 1936, to October 6, 1936; at Woodlake from December 4, 1935, to July 21, 1936. Each interval between collections was approximately 6 weeks. Five collections were made at Oasis and six each at Corona and Woodlake. At Oasis the collecting extended over a period of 168 days, at Corona 210 days, and at Woodlake 211 days. At each collection sufficient fruit was collected to provide 50 fruits for each storage room and 50 for immediate sampling. The fruit was collected from the same group of trees at each picking. Of each lot of 50 fruits 20 were used for the analytical work and the remainder for the respiration studies. The analytical material consisted of the flavedo and albedo from the stem end of the fruit. These lots were ground in a food chopper with a nut-butter attachment, mixed thoroughly, and samples weighed out for pectin, naringin, and water determinations. Duplicate 25-gm. samples were used for the pectins, duplicate 10-gm. samples for naringin and for the water content of the flavedo, and single 50-gm. samples for the water content of the albedo.

Pectic substances were determined as calcium pectate by the method used in work previously reported (**6**). Naringin was determined by the colorimetric method described by HARVEY and RYGG (**5**). Water content of the flavedo was determined by the toluene method and of the albedo by drying to constant weight at 80° C. The latter method was unsatisfactory for the flavedo because of the error introduced by the volatile oils.

The method used in the respiratory studies was that described by HARVEY and RYGG (**7**). In this method the fruit is placed in an airtight chamber fitted with a mercury manometer and the changes in pressure are recorded at frequent intervals. A blank with approximately the same free air space is observed simultaneously in order to provide corrections for barometric pressure changes and for changes in gas volume due to the slight temperature

variations. This correction would not compensate for any changes in rate of respiration brought about by temperature changes.

At the time the fruit is placed in these chambers it is surrounded by atmosphere of normal composition, but as the oxygen is consumed and CO_2 is given off there is a gradual change to anaerobic conditions. During this change the pressure is being reduced.[1] The maximum negative pressure occurs at approximately the time when the O_2 becomes exhausted; then with continued CO_2 production the pressure increases at a fairly constant rate for a considerable period of time. The rate at which these changes in pressure occur at a given temperature depends on the condition of the fruit. It was found that with grapefruit there was a correlation between the length of time during which the pressure remained negative and the pitting which developed after subsequent storage of corresponding lots.

In all except rare instances respiration in stored fruit produced a negative pressure of varying intensity and duration followed by the development of positive pressure at a uniform rate for a period of days. The data presented are based on the duration of this negative pressure.

The storage rooms were maintained at 38°, 46°, 56°, and 68° F. Fruit from Oasis was not stored at 68°. No pectic analyses were made on stored Woodlake fruit nor on the stored fruit of the second picking from Corona.

Results and discussion

Behavior of fruit in storage

The relation between the stored and field lots is shown in figure 1, and the severity of the pitting in tables I and II. Pitting was most severe in the

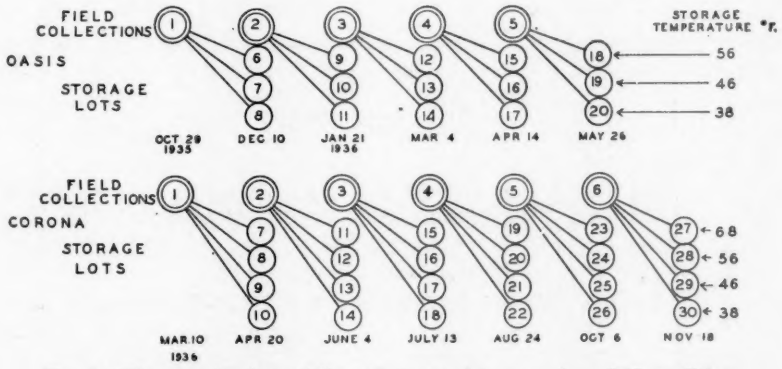

FIG. 1. Diagram showing relation of storage lots to various field conditions.

[1] This apparent reduction in gas volume is accounted for, at least in a large part, by the evolved CO_2 going into solution in the fruit juice. This fact magnifies the need for a standard and constant temperature in using this method.

TABLE I

SEASONAL VARIATIONS IN SUSCEPTIBILITY OF MARSH GRAPEFRUIT TO PITTING AND IN COMPOSITION OF THE RIND WITH REFERENCE TO WATER, NARINGIN, AND PECTIC SUBSTANCES. OASIS, CALIFORNIA, 1935–36*

Date of picking	Lot no.	Storage temperature	Moisture content		Pectic substances						Naringin	Pitting		
					Flavedo			Albedo						
			Flavedo	Albedo	Soluble	Protopectin	Total	Soluble	Protopectin	Total	Albedo	Slight	Severe	Total
		°F.	%	%	gm.	gm.	gm.	gm.	gm.	gm.	%	%	%	%
1935	1	.....	74.3	72.3	1.5	12.7	14.2	2.5	13.9	16.4	10.57	.....	.....	.....
Oct. 29	6	56	74.1	74.1	1.6	13.7	15.3	2.9	17.8	20.7	11.54	27	0	27
	7	46	72.0	74.1	1.0	12.0	13.0	2.3	17.2	19.5	11.31	36	34	70
	8	38	72.3	73.5	1.0	13.7	14.7	2.3	16.5	18.8	10.86	14	66	80
	2	.....	75.0	75.5	1.6	12.6	14.2	3.1	12.6	15.7	9.06	.....	.....	.....
Dec. 10	9	56	77.9	75.7	1.8	14.3	16.1	2.7	14.0	16.7	13.04	2	0	2
	10	46	74.2	76.3	1.0	11.5	12.5	2.8	14.8	17.6	12.15	4	12	16
	11	38	74.7	76.0	1.3	13.6	14.9	3.7	13.3	17.0	12.70	34	42	76
1936	3	.....	75.6	75.0	1.9	11.3	13.2	3.5	11.0	14.5	10.24	.....	.....	.....
Jan. 21	12	56	77.9	78.2	1.1	11.3	12.4	3.9	14.2	18.1	10.82	.....	.....	.....
	13	46	77.6	77.7	1.2	12.6	13.8	3.7	14.1	17.8	11.34	66	0	66
	14	38	77.6	77.8	1.3	11.2	12.5	4.4	12.4	16.8	11.57	36	22	58
	4	.....	78.8	81.3	1.7	12.5	14.2	5.7	11.3	17.0	10.90	.....	.....	.....
Mar. 4	15	56	80.5	80.2	2.0	12.9	14.9	5.2	12.7	17.9	12.37	16	0	16
	16	46	79.1	81.2	1.7	12.5	14.2	5.4	10.7	16.1	12.10	72	6	78
	17	38	80.7	80.9	2.0	13.5	15.5	6.3	9.5	15.8	10.94	4	96	100
	5	.....	81.0	80.6	1.7	11.9	13.6	6.1	11.6	17.7	10.46	.....	.....	.....
Apr. 14	18	56	79.1	80.7	1.1	11.4	12.5	5.3	12.7	18.0	13.09	8	0	8
	19	46	78.0	80.3	1.7	12.4	14.1	3.2	14.8	18.0	12.33	40	40	80
	20	38	77.7	80.6	1.5	14.0	15.5	4.5	13.4	17.9	10.25	14	86	100

* Moisture is expressed in percentage of fresh weight, naringin in percentage of dry weight, and pectic substances in grams of calcium pectate per 100 gm. of dry tissue.

TABLE II

SEASONAL VARIATIONS IN SUSCEPTIBILITY OF MARSH GRAPEFRUIT TO PITTING AND IN COMPOSITION OF THE RIND WITH REFERENCE TO WATER, NARINGIN,

TABLE II

SEASONAL VARIATIONS IN SUSCEPTIBILITY OF MARSH GRAPEFRUIT TO PITTING AND IN COMPOSITION OF THE RIND WITH REFERENCE TO WATER, NARINGIN, AND PECTIC SUBSTANCES. CORONA, CALIFORNIA, 1935–36*

Date of picking	Lot No.	Storage temperature	Moisture content		Pectic substances						Naringin	Pitting		
					Flavedo			Albedo						
			Flavedo	Albedo	Soluble	Protopectin	Total	Soluble	Protopectin	Total	Albedo	Slight	Severe	Total
		°F.	%	%	gm.	gm.	gm.	gm.	gm.	gm.	%	%	%	%
1936	1	.....	77.0	78.2	1.4	10.6	12.0	6.0	9.8	15.8	8.90	.....	.....	.....
March 10	7	68	79.4	79.3	1.8	12.5	14.3	7.3	11.1	18.4	11.78	4	0	4
	8	56	78.3	78.2	1.6	13.2	14.8	6.6	11.0	17.6	11.69	4	0	4
	9	46	78.9	79.2	1.5	12.6	14.1	6.2	11.3	17.5	11.53	32	8	40
	10	38	79.7	80.0	1.5	12.4	13.9	6.8	10.1	16.9	11.90	24	66	90
	2	.....	77.3	80.6	1.4	9.9	11.3	5.6	10.4	16.0	10.10	.....	.....	.....
	11	68	.....	80.5	.....	.....	.....	.....	.....	.....	12.20	0	0	0
April 20	12	56	.....	79.8	.....	.....	.....	.....	.....	.....	11.48	0	0	0
	13	46	.....	80.0	.....	.....	.....	.....	.....	.....	10.50	58	10	68
	14	38	.....	80.1	.....	.....	.....	.....	.....	.....	10.65	26	62	88
	3	.....	79.4	80.1	1.5	10.5	12.0	5.9	8.6	14.5	9.90	.....	.....	.....
	15	68	81.6	81.8	2.6	13.1	15.7	8.1	9.9	18.0	12.25	0	0	0
June 4	16	56	77.5	81.4	1.8	10.2	12.0	6.7	10.6	17.3	12.63	6	0	6
	17	46	80.3	79.4	1.8	13.3	15.1	6.9	9.3	16.2	10.09	48	12	60
	18	38	81.0	82.4	1.6	14.7	16.3	6.2	10.7	16.9	12.15	48	32	80
	4	.....	82.0	81.0	1.6	11.6	13.2	6.3	10.7	17.0	11.15	.....	.....	.....
	19	68	81.5	81.0	2.3	14.8	17.1	6.8	11.3	18.1	12.21	0	0	0
July 13	20	56	79.7	80.8	2.0	12.8	14.8	6.1	11.0	17.1	11.51	0	0	0
	21	46	80.4	82.1	2.1	14.1	16.1	3.8	12.8	16.6	12.12	76	12	88
	22	38	81.2	80.7	1.8	15.4	17.2	4.8	11.7	16.5	11.76	22	76	98
	5	.....	79.8	81.1	1.3	12.8	14.1	5.7	11.7	17.4	10.63	.....	.....	.....
	23	68	81.8	80.8	2.9	14.5	17.4	6.3	12.6	18.9	12.76	0	0	0
Aug. 24	24	56	77.0	79.6	2.2	10.5	12.7	6.4	13.4	19.8	12.79	4	0	4
	25	46	79.8	80.3	1.4	12.9	14.3	3.9	13.9	17.8	12.33	58	4	62
	26	38	79.8	80.4	1.6	14.3	15.9	5.3	11.4	16.7	11.42	44	54	98
	6	.....	81.7	80.7	2.5	14.3	16.8	7.4	11.6	19.0	11.96	.....	.....	.....
	27	68	81.5	82.3	1.3	11.9	13.2	7.8	10.2	18.0	11.41	2	0	2
Oct. 6	28	56	81.3	80.4	2.0	11.8	13.8	5.7	12.6	18.3	11.17	2	0	2
	29	46	82.1	81.1	2.0	12.6	14.6	5.1	13.4	18.5	11.85	82	0	82
	30	38	76.6	76.8	1.1	9.1	10.2	6.8	13.9	20.6	9.27	44	56	100

* Moisture is expressed in percentage of fresh weight, naringin in percentage of dry weight, and pectic substances in grams of calcium pectate per 100 gm. of dry tissue.

fruit stored at 38° F. regardless of the source. Figure 2 shows the results obtained with fruit from Oasis, and figure 3 the results with fruit from

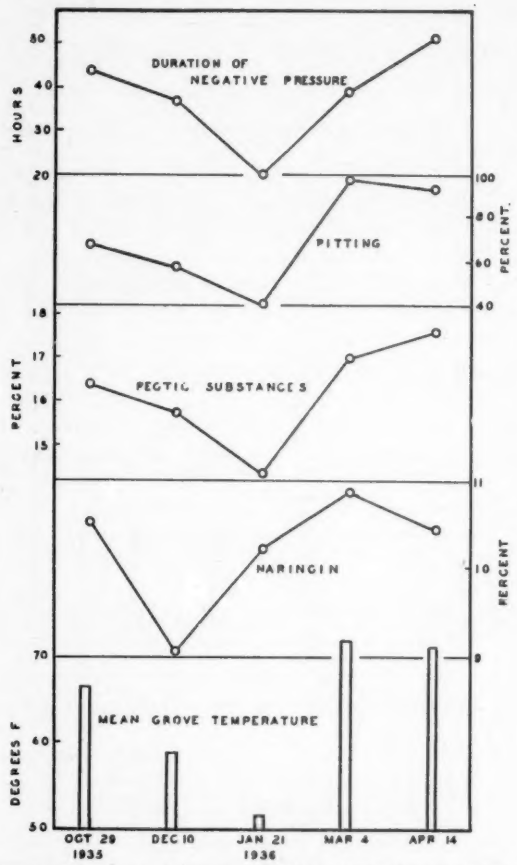

FIG. 2. Hours of negative pressure, weighted pitting percentage, total pectic content, and percentage of naringin of the albedo of the stem end of Marsh grapefruit grown near Oasis, California, with mean temperatures for the 5 days preceding picking.

Corona. The values used to express the severity of pitting were obtained by adding the percentage of severely pitted and one-half the percentage of slightly pitted fruit. This evaluation is arbitrary but was adopted for convenience. The grading of the pitting is also arbitrary; it is the same as that used in the previous report (6), namely, that pitting which was considered of commercial importance was classified as severe and anything less as slight.

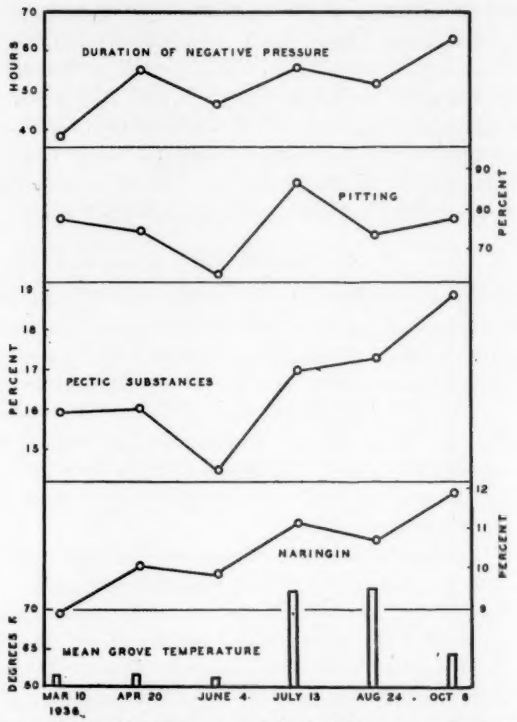

FIG. 3. Hours of negative pressure, weighted pitting percentage, total pectic content, and percentage of naringin of the albedo of the stem end of Marsh grapefruit grown near Corona, California, with mean temperatures for the 5 days preceding picking.

The fruit from Oasis pitted most severely early and late in the season with a period of reduced susceptibility in midseason. The susceptibility to pitting varied with the temperature prevailing previous to picking, being higher with the higher temperatures as shown in figure 2. The temperatures given are the averages of the mean daily temperature during the 5 days preceding the picking. Similar results were obtained with grapefruit from Oasis in 1933–34 and 1934–35, and from Phoenix, Arizona, during the season of 1934–35.

The fruit from Corona pitted with varying degrees of intensity during the season, but the least pitting also occurred about midseason at this location. There was considerable agreement between the mean temperature of the 5 days preceding picking and the susceptibility to subsequent pitting.

The fruit from Woodlake was highly resistant to pitting early in the season and became progressively more susceptible as the season advanced

until the last two pickings, when every fruit pitted and nearly all pitted severely. The first picking showed only 18 per cent. of slight pitting and no severe pitting after 6 weeks at 38° F. These results are in marked contrast with those obtained on fruit from Oasis which, if picked early, showed a high degree of susceptibility to pitting when stored at 38°. The relationship of pitting to the temperatures preceding picking was not so marked as with the Oasis fruit, although the lowest temperatures occurred previous to the first three pickings when the fruit was least susceptible to pitting.

Respiration

It was found that the length of time during which the pressure remained negative differed considerably with fruit from the different groves; *e.g.*, this period of negative pressure developed by unstored fruit varied from 20 to 44 hours with fruit from Oasis, 40 to 68 hours with fruit from Corona, and from 17 to 82 hours with that from Woodlake. While the relationship of time of negative pressure to susceptibility to subsequent pitting holds roughly for fruit from an individual grove or group of trees, it does not hold when the behavior of fruit from one location is compared with that from another, perhaps many miles distant.

Fruit which had been stored and subsequently removed and placed in the chambers showed a speeding up of the metabolic processes which brought about these changes in pressure. This acceleration was proportional to the lowering of the storage temperature and the length of time during which the fruit had been held at these temperatures. It was also found that the rate as measured by the duration of the negative pressure was more rapid

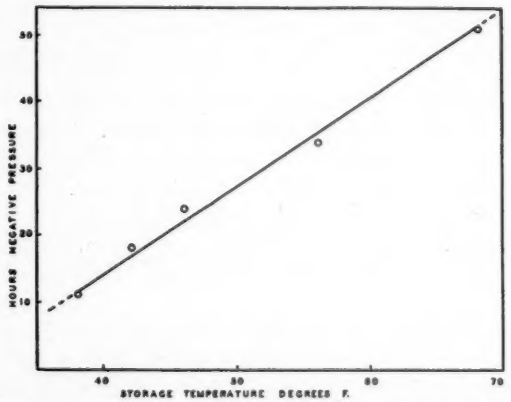

Fig. 4. The duration of the period of negative pressure developed by grapefruit in airtight chambers at room temperature subsequent to 6-weeks' storage at the temperature indicated.

in fruit which had pitted severely than in fruit from the same lots which had been held at the same temperature for the same period of time but which had pitted less severely or not at all. Figure 4 shows the relationship between the storage temperature and the hours of negative pressure developed by the fruit at room temperature. Each point on the graph represents the average of from 15 to 50 chambers.

The effect of the length of storage on the rate of subsequent anaerobic respiration at room temperature as measured by the duration of the negative pressure is shown in figure 5.

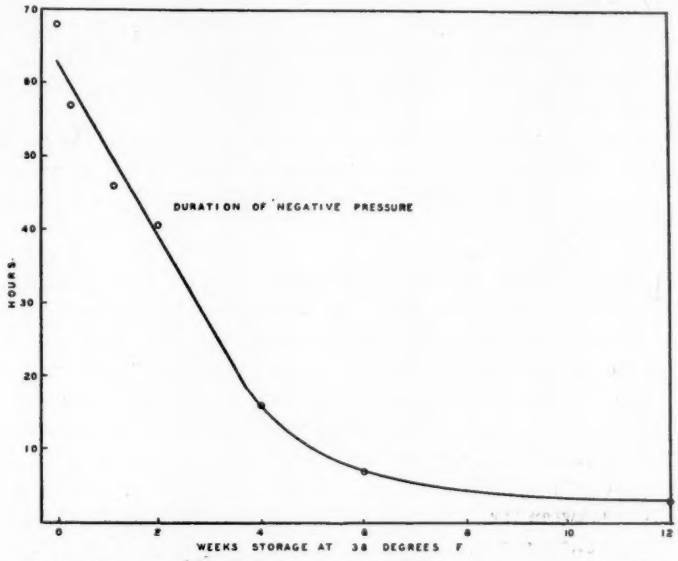

FIG. 5. The effect of length of storage period on the duration of the negative pressure developed. Storage at 38° F.

The fruit was placed in storage at 38° F. immediately after picking, except for one portion which was taken to the laboratory for immediate testing. The remainder of the fruit was removed from storage after intervals as indicated in figure 5, brought to the laboratory, and placed in the chambers. The duration of the period of negative pressure began to be reduced immediately after the fruit was stored. After 2 days' storage the period was reduced from 68 hours to 57 hours; additional tests were made after 8 days, and 2, 4, 6, and 12 weeks. The duration of the period of negative pressure varied inversely with the length of the storage period until pitting set in and the period of negative pressure had been reduced to a relatively

short time. After 4 weeks in storage the fruit had developed pitting to the extent of 18 per cent. severely pitted and 76 per cent. slightly pitted. All the fruit was severely pitted after 6 weeks in storage.

Pectic substances

Changes in pectic contents in the field during the harvest season.—*Soluble pectin.*—The soluble pectin in the flavedo of the fruit from Oasis showed little change during the season. At the beginning of the season (October 29) there were 1.5 gm. of Ca pectate per 100 gm. of dry tissue; this increased to 1.9 gm. in midseason (January 21) and fell to 1.7 gm. at the end of the season (April 14). Soluble pectin in the albedo of the same fruit increased gradually throughout the season from 2.5 to 6.1 gm. This increase agrees with results obtained by Gaddum (**4**) on albedo of citrus fruit grown in Florida.

Soluble pectin in the flavedo of grapefruit grown at Corona increased from 1.4 gm. per 100 gm. of dry tissue on March 10 to 2.7 gm. on October 6. In the albedo the soluble pectin fluctuated somewhat during the season; the increase was from 6.0 to 7.4 gm.

In the flavedo of fruit from Woodlake the soluble pectin increased from 1.1 gm. on December 4 to 2.4 gm. on May 20, and dropped to 2.0 gm. by July 2. In the albedo of this fruit the soluble pectin increased throughout the season from 2.1 gm. on December 4 to 5.2 gm. on July 2.

In all instances the trend was for soluble pectin to increase during the season, though sometimes the increase was slight, especially in the flavedo. The albedo contained far more soluble pectin than the flavedo, the average of all field samples from Oasis being 4.2 and 1.7 gm.; from Corona 6.0 and 1.5 gm.; and from Woodlake 3.7 and 1.6 gm., respectively. There was no relationship between the soluble pectin content and the susceptibility of the grapefruit to pitting at any temperature.

Protopectin.—The protopectin content of the flavedo of fruit from Oasis varied somewhat, with the minimum occurring at the time of the third collection and the maxima at the beginning and the end of the picking season. In the albedo the minimum was also in the third collection and the maximum was in the first.

The protopectin in the flavedo of the fruit from Corona increased as the season progressed, with the minimum at the time of the second picking and the maximum at the end of the picking season. In the albedo the minimum occurred at the time of the third picking.

The protopectin in the flavedo of the fruit from Woodlake decreased throughout the season, falling from 13.8 gm. at the time of the first picking to 9.0 gm. at the end of the season. There was more fluctuation in the protopectin of the albedo, but there was no seasonal trend.

There was no trend in the changes in protopectin, which was true for both parts of the rind and for fruit collected from all locations. There was, however, a tendency for the minimum to occur at about midseason, especially in the albedo. This variability is not surprising in view of the variations in the climatic environment of the fruit from the different districts. It has already been shown that the climatic environment of citrus during its growth and immediately preceding its harvest has marked effects on the response of the fruit to treatments given after harvest. There appears to be a relationship between the protopectin of the rind, especially of the albedo, at the time of picking and the subsequent behavior of the fruit in storage, but the relationship between the total pectic content and the storage response is more apparent and will be discussed under that heading. The protopectin of the flavedo and albedo were practically equal. The average values for all the field collections were: Oasis, flavedo 12.2 gm., albedo 12.1 gm.; Corona, flavedo 12 gm., albedo 10.5 gm.; Woodlake, flavedo 11.6 gm., albedo 11.4 gm.

Total pectic substances.—The amount of total pectic substances in the flavedo of grapefruit from Oasis was lowest during midseason but the variation during the season was slight, ranging from 13.2 to 14.2 gm. There were considerably less total pectic substances present in the albedo during midseason than either before or after, the range in this tissue being from 14.4 to 17.7 gm. Figure 2 shows the amount at each picking and the relation to the mean temperature for the last 5 days before picking, respirational behavior, naringin content, and susceptibility to pitting.

The amount of total pectic substances of the flavedo of grapefruit from Corona increased from the second to the last collection, with the contents varying from 11.3 to 16.8 gm. There was more fluctuation in the total pectic substances in the albedo, where the minimum occurred in midseason and the maximum at the end of the season. The amount of total pectic substances in this tissue ranged from 14.5 to 18.9 gm. These fluctuations were apparently in response to environmental changes, as they coincided with the response of the fruit to storage conditions and to the respiratory behavior in tight chambers. These results are shown in figure 3.

The total pectic substances in the flavedo of fruit from Woodlake decreased during the season from 14.9 to 11.0 gm. In the albedo, however, the first two pickings gave the lowest yields of pectic substances and the last four the highest. The values ranged from 12.3 to 16.6 gm. The total pectic substances in the grapefruit from Woodlake did not correspond so well with the subsequent behavior of the fruit as did that from Oasis and Corona but it did agree in that in the albedo the lowest total pectic content and the least susceptibility to storage pitting occurred simultaneously, as did the highest pectic content and greatest susceptibility to pitting.

The nature of the relationship between the total pectic content of the

albedo and susceptibility to storage pitting is not known. At any rate, the response of the fruit on the trees to the environmental conditions were such that conditions which brought about a reduction in the total pectic substances in the albedo also brought about such changes as would reduce the susceptibility of the fruit to pitting at low temperatures. These results indicate that the condition and composition of the albedo at the time of picking are important in determining whether or not the fruit is likely to develop pitting if held at low temperatures.

The amount of total pectic substances in the flavedo held a similar relationship to subsequent pitting but the yield was less variable, hence the differences failed to show so distinctly. The flavedo of the fruit from Woodlake did not give results agreeing with the others as the pectic content decreased during the entire season.

The pitting resulting after 6 weeks storage at 46° F. also agreed with the results mentioned above, but the severity of the pitting was reduced at this temperature.

A comparison of the total pectic contents of the flavedo and albedo shows the albedo to be consistently but not greatly higher. Average values for all field collections from the various districts are: Oasis, flavedo 13.8 gm., albedo 16.3 gm.; Corona, flavedo 13.7 gm., albedo 16.7 gm.; Woodlake, flavedo 13.2 gm., albedo 15.1 gm.

CHANGES IN THE PECTIC CONTENTS OF STORED FRUIT.—The relationship in the proportion of soluble pectin to protopectin did not change materially in grapefruit stored 6 weeks at the temperatures mentioned. A surprising result, however, was the frequent apparent increase in total pectins at the end of these storage periods. Table III shows the average pectic content of the flavedo and albedo for a total of ten storage lots for each temperature

TABLE III

TOTAL PECTIC SUBSTANCES IN THE RIND OF THE STEM END OF MARSH GRAPEFRUIT BEFORE STORAGE AND AFTER STORAGE FOR 6 WEEKS, EXPRESSED AS GRAMS Ca PECTATE PER 100 GM. DRY TISSUE

	No storage	Storage			
		68° F.*	56° F.	46° F.	38° F.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
Flavedo	13.7	15.5	13.9	14.2	14.7
Albedo	16.5	18.3	18.1	17.6	17.4

* Figures in this column are for the fruit from Corona only, and represent the average of 5 pickings; all other values are for the fruit from Corona and Oasis, and represent a total of 10 pickings.

given and a corresponding number of fruit sampled immediately after collection.

In every instance the value for the stored lot was higher than that for the corresponding unstored lot. Also, there was a tendency for the value to be highest for the fruit stored at the highest temperature. BUSTON (3) indicates that high water content favors pectin production in growing tissue. No such condition is indicated in this instance, as the water content of these lots of fruit show very little variation, being 78.5 per cent. for both the flavedo and albedo of the field collections and 78.4, 78.3, and 77.7 per cent. in the flavedo and 78.9, 79.2, and 79.0 per cent. in the albedo of the fruit stored at the temperatures of 56°, 46°, and 38° F., respectively. Furthermore, this was not growing tissue. According to ONSLOW (8), however, the production of protopectin is brought about in steps by the inversion and oxidation of glucose and the entire system is in a state of flux. The reactions within the system are probably controlled by the "level of oxidative activity" of the cells (1). Consequently the direction and magnitude of the reactions are dependent on the factors that control this level. The apparent increase in total pectic content was brought about principally by increases in the protopectin fraction; out of 70 samplings there was an increase in soluble pectin in 41 instances, or 59 per cent. of the lots. In the same number of lots protopectin increased in 54 instances, or 77 per cent. of the lots, and total pectic substances increased in 73 per cent. of the lots. All previous reports of pectic changes of stored fruit have shown decreases. No doubt this would have occurred in grapefruit provided the fruit had been held long enough. Figures presented by BRANFOOT (Carré) (2) show that in apples the fruit was stored 17 weeks before the total pectic content began to decrease. No such long storage periods have been attempted in the present studies on pectic substances in grapefruit. BRANFOOT (2), however, found that pectose began to decrease and the soluble pectin to increase immediately after the fruit had been placed in storage, but this behavior has not been noted for grapefruit rind.

While all the pectin analyses are reported on the basis of dry weight, the same situation existed when the pectic contents were expressed on the basis of fresh weight.

NARINGIN

The behavior of naringin in mature or nearly mature grapefruit which remains attached to the tree does not follow a definite seasonal trend. In the fruit from Oasis the naringin content of the stem-end albedo[2] followed roughly the mean temperatures for the 5-day period preceding picking, with

[2] Naringin was not determined in the flavedo because the pigments in that tissue interfered with measurements by the colorimetric method used.

the minimum at midseason. However, the naringin minimum occurred at the second picking while the minimum temperature occurred at the time of the third picking (fig. 2). A similar temperature relationship was reported previously for the season of 1933–34 (**6**).

The naringin content of the albedo of fruit from Corona similarly followed roughly the changes in the mean temperature before picking. In this instance there was an increase until the end of the season (fig. 3). This seasonal trend is opposite to that recorded previously for fruit of another season from the same grove, but the temperature relationship is the same as was obtained during that season.

It will be noted in figures 2 and 3 that there is a relationship between the naringin content and the susceptibility to storage pitting. It does not follow that the presence of naringin influenced the fruit in its susceptibility to pitting but merely indicates, as was stated in the discussion of pectins, that changes take place in the composition of the fruit while it is still attached to the tree, and that these changes influence the susceptibility of the fruit to storage pitting.

The naringin content of the albedo of grapefruit from Woodlake was lowest at the time of the first picking (December 4), increased to a maximum at the time of the fourth (April 8), and subsequently decreased until the end of the season (July 2). The naringin content of the albedo of Woodlake fruit did not vary according to the temperatures preceding picking, neither did its variations coincide with those in pectic content.

In an earlier paper the fact was mentioned that the change in naringin content of stored grapefruit was controlled by factors that were poorly understood and that it might increase or decrease depending upon the condition of the fruit as well as on the conditions of storage. Present work corroborates this point of view. It also indicates a preponderant tendency for the naringin content to increase in storage through a wide range of storage temperatures with a tendency for a greater increase in fruit held at

TABLE IV

NARINGIN CONTENT OF STEM-END ALBEDO OF FRESH AND STORED GRAPEFRUIT FROM OASIS, CORONA, AND WOODLAKE, CALIFORNIA

LOCATION	NO STORAGE	STORAGE			
		68° F.	56° F.	46° F.	38° F.
	%	%	%	%	%
Oasis (dry weight basis)	10.25	.....	12.17	11.85	11.26
Oasis (fresh weight basis)	2.36	.....	2.71	2.60	2.52
Corona (dry weight basis)	10.44	12.10	11.88	11.40	11.19
Corona (fresh weight basis)	2.05	2.31	2.34	2.24	2.22
Woodlake (dry weight basis)	8.03	.....	9.60	8.94	8.74
Woodlake (fresh weight basis)	1.64	.....	1.90	1.76	1.70

the higher temperatures. The average values for fruit stored 6 weeks at the various temperatures are given in table IV.

The differences are not great, especially when expressed on the fresh weight basis, but the same is true whether the percentages are expressed on the fresh or the dry weight basis.

The results reported in this paper give support to the suggestion made in the earlier report that naringin might be an indirect product of respiration, as both in the field and in storage there was a tendency for the naringin content to be higher following exposure to higher temperatures.

Summary

1. Results are presented on the effect of season and storage temperature on the pitting developed by Marsh grapefruit and on certain chemical changes taking place in the rind of the fruit. Fruit for the study was grown in three widely separated citrus districts in California: (1) Oasis, in the Coachella Valley in the interior portion of southern California; (2) Corona, in the western portion of southern California; and (3) Woodlake, in the San Joaquin Valley.

The storage temperatures used were 38°, 46°, and 56° F. Pitting was always most severe in fruit stored at 38° and least in that stored at 56° F.

Fruit from Oasis pitted most severely at the beginning and the end of the harvest season, the severity varying directly with the mean temperature for the five days preceding picking.

Fruit from Corona pitted with varying degrees of intensity during the season, but the pitting held approximately the same relationship to the mean temperatures before picking as that from Oasis.

Fruit from Woodlake was highly resistant to pitting early in the season and became progressively more susceptible as the season advanced. There was some relationship between susceptibility to pitting and the mean temperatures preceding picking but not as marked as with fruit from Oasis and Corona.

2. Respiration of the fruit in sealed chambers was measured by the duration of the period of negative pressure. There was a direct relation between the length of this period and the susceptibility of the fruit to pitting. This relation was found to obtain in comparing fruit picked from a given group of trees in a grove, but not in comparing fruit from groves in widely separated regions.

The duration of the period of negative pressure developed by stored fruit varied according to the temperature and duration of the storage, both low temperatures and long storage periods tending to shorten the period of negative pressure. Pitted fruit had a shorter period of negative pressure than unpitted fruit from the same lot.

3. Soluble pectin in the albedo increased during the season.

Changes in protopectin did not follow a seasonal trend but there was a tendency for the minimum to occur at midseason. The changes were apparently influenced by the climatic environment.

Total pectic substances were lowest at midseason in fruit from Oasis and Corona and early in the season in the albedo of fruit from Woodlake. The variations in the albedo were such as to coincide with susceptibility to storage pitting, low pectic substances occurring at the time of low susceptibility, but no causal relationship is postulated.

The total pectic content of the albedo is somewhat higher than that of the flavedo.

Storage of grapefruit for 6 weeks did not change the quantitative relationship between the soluble pectin and protopectin.

There was an apparent slight increase of pectic substances in both the flavedo and the albedo of stored grapefruit.

4. Naringin in the albedo of grapefruit from the field varied in much the same way as the total pectic substances.

In storage naringin usually increased, and the increase was greatest at the higher temperatures.

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AUXIN PRODUCTION IN SEEDLINGS OF DWARF MAIZE[1]

J. VAN OVERBEEK

(WITH EIGHT FIGURES)

Introduction

Dwarf races of corn are mainly characterized by the length of stem which is, at vegetative maturity, less than 50 per cent. of the length of the normal sibs. It is known that the growth in length of stems, and more especially the elongation of the cells, is regulated by auxin (**11**). In an earlier paper on dwarfism (**8**) I stated that *nana* corn, one of the genetic types of dwarfs, shows its dwarfism even in the seedling stage by its reduced growth of the mesocotyl. It was shown that this reduced growth was attributable to inhibited elongation rather than to a reduced number of cells. Recently ABBE (**1**) showed that in mature plants of *nana* and *dwarf-1* the number of internodes is the same as in their normal sibs. It is the shorter length of the cells in the ground parenchyma, as well as in the vascular tissue, which accounts for the short stems of the dwarfs. It was demonstrated (**8**) that 5 days after they were sown in sand in a physiological darkroom, *nana* plants produce less auxin than do normal ones. It was also shown that the sensitivity to auxin (measured as auxin curvatures) is less in the *nana* coleoptiles than in the normal ones. Both differences were attributed to the greater destruction of auxin in the *nana* plants, which was found to be especially high in the mesocotyls. The destruction was thought to be caused by the action of peroxidases, which were found to be much more active in the apical part of the mesocotyl of *nana* plants than of normal corn.

In 1936 a few studies on other plants appeared in which an attempt was made to link dwarfism with auxin. HINDERER (**6**) showed that hybrids of *Epilobium* have an auxin production which is smaller the more dwarfed the hybrid is. DE HAAN and GORTER (**4**) studied a tall and a shorter variety of *Pisum* and concluded that the destruction of auxin is the determining factor for the growth of the pea stem, thereby reaching the same conclusion as I had reached for the *nana* dwarf in corn. (**8**).

The purpose of the present investigation was to study the auxin production in several of the known genetic dwarf races of corn. In order to have comparable controls, seeds were used which segregated either 50 or 25 per cent. dwarfs. The dwarfs of the types here described were easily distinguished from normal control plants 4 days after they had been sown in the darkroom. The seeds were sown and the plants were grown in pure sand (without nutrient solution). The experiments were carried out in the same darkroom where the plants were grown. The temperature was 24° C. and

[1] Second communication on the physiological basis of dwarfism.

the humidity 90 per cent. The plants were occasionally exposed to the orange light of the darkroom lamps.

Experimentation

Factors affecting the amount of auxin given off by coleoptile tips

The production of auxin was determined by the diffusion method. The tips of the coleoptiles were cut off and placed on agar blocks. The auxin produced in the tips was given off into the agar. The auxin content of the agar was then determined by means of the standard *Avena* test (**3, 10, 11**). The yield of auxin obtained in this way is dependent upon the following conditions.

(a) Ridding cut surface of destructive enzymes.—It was found that the yield of auxin could be markedly increased by standing the tips on wet filter paper before putting them on the agar blocks. Coleoptile tips were placed on wet filter paper for 0, ½, 1, and 1½ hours before they were put on

TABLE I

Amount of auxin (in degrees of curvature) diffused from coleoptile tips into agar blocks. After the tips were cut they were placed on wet filter paper before being put on the agar blocks. Normal corn. 60529

Time on wet filter paper	Age of corn seedling in days		
	4	6	7
hr.			
0	4.8*	6.8	3.4
½	5.0	8.3	9.7
1	7.4	12.0	8.7
1½	7.5	12.3	9.0

* Averages of 12 plants, tips, etc. The same holds for the other tables and graphs unless the contrary is stated.

agar blocks (table I). It is clear that the yield increased with the time the cut surface was "washed." The maximum effect is reached by standing for 1 hour on wet filter paper. The effect of standing on wet filter paper is readily explained by assuming that by this treatment the cut surface is cleared from enzymes having a destructive action on auxin. It was shown (**9**) that a cut surface, after it had been in contact with wet filter paper for some time, had a much lower peroxidase activity than a freshly cut surface. In most experiments described in this paper the coleoptiles were put for 1 hour on wet filter paper before they were placed on the agar blocks.

(b) Duration of auxin production.—Isolated coleoptile tips are able to give off auxin at the same rate for at least several hours. Table II shows

TABLE II

AMOUNT OF AUXIN (IN DEGREES OF CURVATURE) GIVEN OFF BY 5-MM. TIPS OF 5-DAY-OLD COLEOPTILES. THE TIPS WERE PLACED ON FRESH AGAR BLOCKS EVERY 45 MINUTES. 60309

VARIETY OF CORN	SUCCESSIVE PERIODS OF 45 MINUTES					
	1	2	3	4	5	6
Normal	15.5	17.0	16.0	18.0	16.0	13.1
Nana	9.0	9.2	10.6	7.6	8.7	8.7

the results of an experiment with tips of 5-day-old *nana* and normal plants. The tips were placed on fresh agar blocks each 45 minutes for 5 successive times. The amounts of auxin diffusing into the blocks were practically constant during the time of the experiment.

(c) LENGTH OF THE TIP.—The length of the tip does not greatly affect the amount of auxin given off. The 5-mm. tips used in the experiment recorded in table II continued to give off auxin at the same rate for 4½ hours. In table III results of two experiments with tips 1 and 2 mm. long are presented. These short tips, too, continued to give off auxin for 4½ to 6 hours at practically the same rate. These experiments show that the production of auxin by the extreme tip, when isolated, may continue for a considerable time.

TABLE III

AMOUNT OF AUXIN (IN DEGREES OF CURVATURE) GIVEN OFF BY COLEOPTILE TIPS OF ABOUT 5-DAY-OLD SEEDLINGS DURING SUCCESSIVE PERIODS OF 1½ HOUR

VARIETY OF CORN	LENGTH OF TIP	EXPERIMENT NUMBER	SUCCESSIVE PERIODS OF 1½ HR.			
			1	2	3	4
Normal	2 mm.	41024	18.6	17.1	19.0	
Nana	2 mm.		11.0	13.5	15.9	
Normal	1 mm.	41025	15.0	6.0	11.0	12.0
Nana	1 mm.		8.0	7.0	6.0	7.0

(d) AGE OF THE SEEDLING.—The age of the seedling is an important factor in determining the amount of auxin given off by the coleoptile tips. In figure 1 the age of the seedling (days after the seeds were planted in the sand) is plotted against the production of auxin. The length of the tips was 5 mm., a length which was maintained for most of the tips used in experiments described in this paper. The tips were placed for 1 hour on the agar blocks. The amount of auxin given off is expressed in degrees of curvature obtained in the standard *Avena* test. A control test with blocks con-

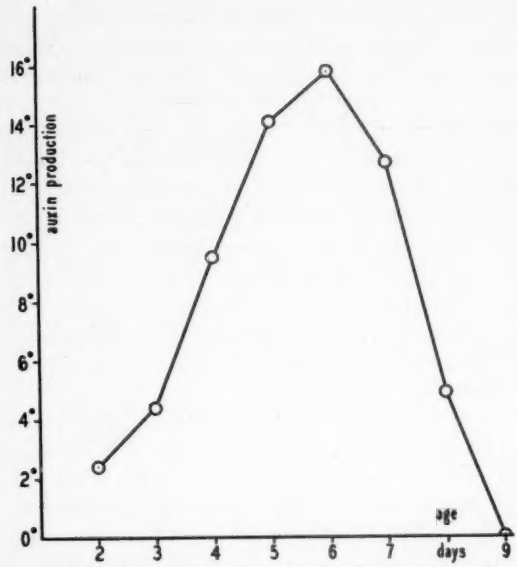

FIG. 1. The relation between the auxin production of the coleoptile tip and the age of the seedling in a normal corn strain (60316). At the stage of optimal production an amount of 2×10^{-10} gram of auxin per hour per tip is given off.

taining a known concentration of indole-acetic acid showed that a curvature of 8.9° was obtained if the concentration in the agar blocks was equivalent to 64.5 gamma per liter. The blocks on which the corn tips had been standing contained auxin-a or auxin-b, which are according to KÖGL and KOSTERMANS (7), twice as active by weight as indole-acetic acid. Hence, the concentration of auxin-a or auxin-b in the block on which one tip of a 4-day-old coleoptile (figure 1) had been standing for one hour was about 34 gamma per liter. Since the agar block on which one tip was standing had a volume of 4 mm.^{3}, the total amount of auxin-a or auxin-b given off per hour by one tip of a 4-day-old normal corn seedling was 1.36×10^{-4} gamma (1.36×10^{-10} gm.). In corn seedlings the primary leaf will break through the coleoptile on about the fifth day. This means that the auxin production is still large and has not even reached its optimum when the leaf breaks through.

THE AUXIN PRODUCTION OF NANA SEEDLINGS

The *nana* character is inherited as a simple, recessive Mendelian character. The gene is located in the third chromosome. The gene locations are quoted after EMERSON, BEADLE, and FRASER (5).

(a) THE AUXIN CONTENT OF THE SEED.—Large amounts of auxin can be

extracted from corn seeds. The question arose whether seeds producing *nana* plants differ in auxin content from seeds producing normal ones. This was worked out in the following way. Seeds were obtained by back-crossing pure *nana* dwarfs with an F_1 plant. Such seeds will segregate 50 per cent. *nana* plants and 50 per cent. normal ones. The individual grains were ground in a mortar (average weight of 1 seed was 0.125 gm.). The pulverized seed was extracted with 5 cc. of distilled water for about 15 hours at 2 to 3° C. An agar block was soaked in the extract. The agar block was analyzed by means of the standard *Avena* test. The results of these determinations are plotted in figure 2. The auxin content of the seeds

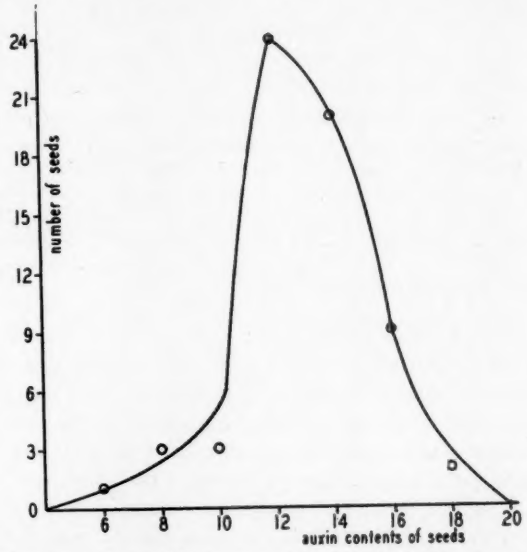

FIG. 2. Frequency curve of the auxin content of individual corn grains of a mixture consisting of 50 per cent. *nana* seeds and 50 per cent. of normal seeds (60114).

was plotted on the abscissa and the number of seeds having a certain auxin content is plotted on the ordinate. The resulting curve is a frequency curve having one single peak, indicating that the *nana* seeds do not differ from the normal ones in regard to auxin content. A control test with other seeds of the same sample showed that 50 per cent. of the germinated seeds developed into dwarfs and the remaining ones into normal corn plants, as could be expected.

(b) PRODUCTION AND GROWTH CURVES.—The production of auxin of the dwarfs has been determined in seedlings from 4 to 7 days old. The lower limit was set because it was practically impossible to distinguish dwarfs

from normal plants at a stage earlier than 4 days after the seeds had been planted. The higher limit of 7 days was chosen because it was shown (figure 1) that the production after this day decreases rapidly. Figure 3a

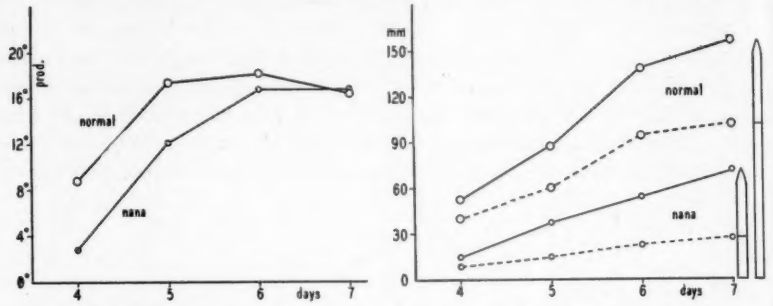

FIG. 3a. Left. Relation between the amount of auxin given off by the coleoptile and the age of the seedling in series segregating *nana* dwarfs. Ordinate: amount of auxin given off by 12 tips, of 5 mm. length, per hour. The amount is expressed in degrees of curvatures in the *Avena* test. Abscissa: age of the seedlings counted from the moment the seeds were planted in sand. Averages of 48 plants (60316, 60502, 60514, 60515).

FIG. 3b. Right. The length of *Nana* plants as compared with normal ones at various stages of development. The dotted lines indicate the position of the coleoptilar nodes, which separate the coleoptile and the mesocotyl.

shows that the production of auxin by *nana* dwarfs is considerably lower than that of the normal sib when the plants are 4 or 5 days old. In older plants, however, the auxin production is practically the same for *nana* and normal plants. The length of the dwarfs and that of the normal plants at the various stages of development are given in figure 3b. It is especially the mesocotyl which grows much more slowly in the *nana* plants than in the normal ones. The fact that the production of auxin in the coleoptile tip of 6- and 7-day-old *nana* plants is the same as that in normal ones does not necessarily mean that the growing zones receive equal amounts of auxin.

TABLE IV

AMOUNTS OF AUXIN GIVEN OFF AT THREE DIFFERENT LEVELS OF CORN SEEDLINGS. PLANTS 6 DAYS OLD. CUT SURFACES WASHED IN WATER FOR 1½ HOURS PRIOR TO DIFFUSION TEST. 60319

Cut surface 5 mm. below coleoptile tip	Normal	49.0°
	Nana	46.6° (5% less than normal)
Cut surface 2 mm. above coleoptilar node	Normal	22.3°
	Nana	17.5° (22% less than normal)
Cut surface 2 mm. below the node	Normal	11.6°
	Nana	1.5° (87% less than normal)

This is shown by the following experiments. Six-day-old plants were divided into three groups. From the plants of the first group (see table IV) 5-mm. coleoptile tips were cut, and the amount of auxin given off by them was determined. It was found that the *nana* plants of this set produced about 5 per cent. less auxin than the normal controls. In the next set the plants were cut off 2 mm. above the node, and by placing agar blocks against the cut surface it could be determined how much auxin, approximately, would reach the basal region of the coleoptile. It was found that in this region the *nana* plants received 22 per cent. less auxin than the normal ones. The plants of the third set were cut off 2 mm. below the coleoptilar node, and the amount of auxin diffusing out of the cut surface was collected in agar. It was found that in the region below the coleoptilar node, which is the growing region of the mesocotyl, 87 per cent. less auxin was present in the dwarfs than in the normal plants. The way the auxin travels from the coleoptile tip to the mesocotyl is for 6-day-old plants shorter for the dwarfs than for the normal plants as follows from figure 3b. Hence, this can not account for the greater disappearance of auxin in the *nana* plants. In a previous paper on *nana* dwarfs it was concluded that the greater destruction in *nana* plants was responsible for their reduced growth. This destruction was determined again for the 6-day-old plants. Sections were cut from the basal part of the coleoptiles in such a way that the basal cut surface was just at the coleoptilar node. Such sections were placed for 3 hours on wet filter paper in order to free them of the auxin they might contain. After that period of time they were placed with their basal cut-surface on agar blocks containing 16.9°-indole-acetic acid. After 1¾ hours the sections were removed and the amount of auxin remaining in the blocks determined. It was shown that 37 per cent. of the auxin was destroyed by the sections of normal corn plants, whereas 92 per cent. was destroyed by the sections of the dwarfs (table V). These facts support the conclusion previously

TABLE V

DESTRUCTION OF AUXIN BY 6-DAY-OLD *nana* AND NORMAL CORN PLANTS. SECTIONS 5 MM. LONG WERE CUT OUT OF THE BASAL PART OF THE COLEOPTILE. THE LOWER CUT WAS THROUGH THE COLEOPTILAR NODE. THE SECTIONS WERE PUT WITH THEIR MORPHOLOGICAL BASE ON WET FILTER PAPER FOR 3 HOURS FOLLOWED BY 1¾ HOURS ON AGAR BLOCKS CONTAINING AUXIN. AVERAGES OF 48 TO 24 PLANTS. 60605

Initial auxin concentration in the agar blocks	16.9°
Left by section of normal corn	10.7° (destroyed 37%)
Left by sections of *nana* corn	1.4° (destroyed 92%)

reached (7) that in the *nana* dwarfs the excessive destruction of auxin is responsible for their reduced growth.

AUXIN PRODUCTION AND GROWTH OF DWARF-1

Figures 4a and 4b present a similar set of curves for *dwarf-1* as the figures 3a and 3b for *nana*. The auxin production of *dwarf-1* remains below

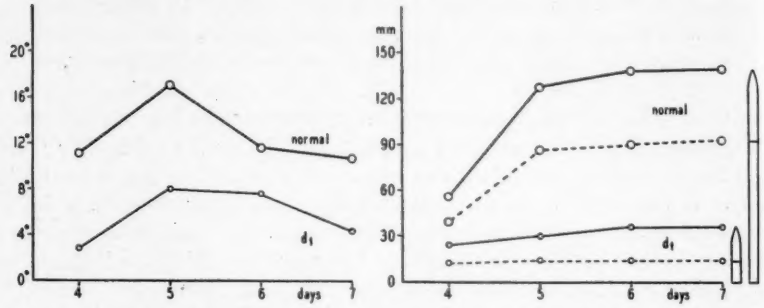

FIG. 4a. Left. Relation between the amount of auxin given off by the coleoptile and the age of the seedling in series segregating dwarf-1. Ordinate and abscissa as in figure 3a. Average values of 36 plants (60415, 60511, 60519).

FIG. 4b. Right. The length of dwarf-1 as compared with normal plants at various stages of development.

that of the normal plant at all stages of development. *Dwarf-1* is a much more extreme dwarf than is *nana*, as well in mature as in seedling stage. Both the coleoptile and the mesocotyl are much shorter in the dwarf than in the normal control plants. The location of the d_1 gene is in the third chromosome as in *nana*, but the genes are located at opposite ends of the chromosome.

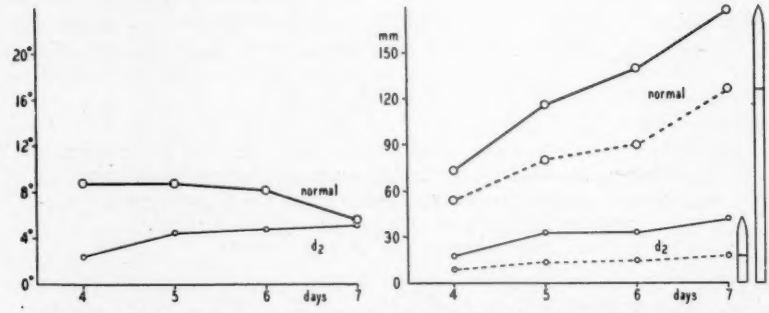

FIG. 5a. Left. Relation between the amount of auxin given off by the coleoptile and the age of the seedling in series segregating dwarf-2. Ordinate and abscissa as in figure 3a. Average values of 36 plants (60408, 60430, 60521).

FIG. 5b. Right. The length of dwarf-2 as compared with normal plants at various stages of development.

Auxin production and growth of dwarf-2

Dwarf-2 is very similar in appearance to *dwarf-1*. The strain used in the experiments had a poor viability. The normal plants were very thin and they grew faster in the early stages of development than does the average corn seedling. In the figures 5a and 5b it is shown that the auxin production of the dwarf is lower than that of the normal plant. The location of the d_2 gene is in the third chromosome.

Auxin production and growth of dwarf-3

Dwarf-3 is also an extreme dwarf like the *dwarf-1* and *dwarf-2*. The stock used was a vigorous one. This dwarf, too, had an auxin production lower than that of the normal sib at all stages of development. The curves shown in figures 6a and 6b are similar to those of *dwarf-1*. The gene is located in chromosome 9.

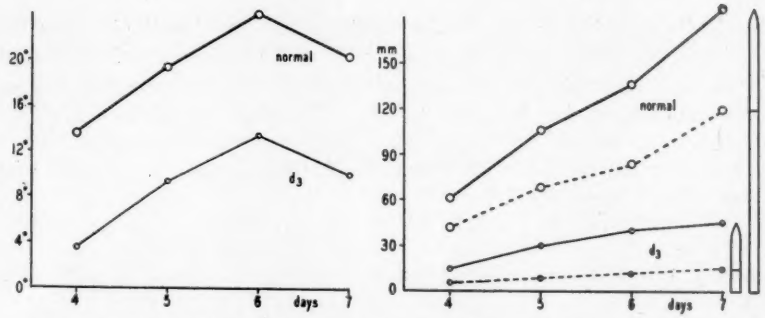

Fig. 6a. Left. Relation between the amount of auxin given off by the coleoptile and the age of the seedling in series segregating dwarf-3. Ordinate and abscissa as in figure 3a. Averages of 36 plants (60520, 60526, 60527).

Fig. 6b. Right. The length of dwarf-3 as compared with normal plants at various stages of development.

Auxin production and growth of dwarf-7

At seedling stage *dwarf-7* is very similar in appearance to *dwarf-1* and the other extreme dwarfs. At vegetative maturity, however, this dwarf is much taller than the other dwarfs, including *nana*, and may reach more than half the length of the plants of the normal sib. Figures 7a and 7b are the growth and auxin production curves for *dwarf-7*, whose gene d_7 is located in chromosome 10.

Auxin production and growth of pigmy

The *pigmy* dwarf is one of quite different type than the ones discussed above. In the field it is characterized by its short and striated leaves rather

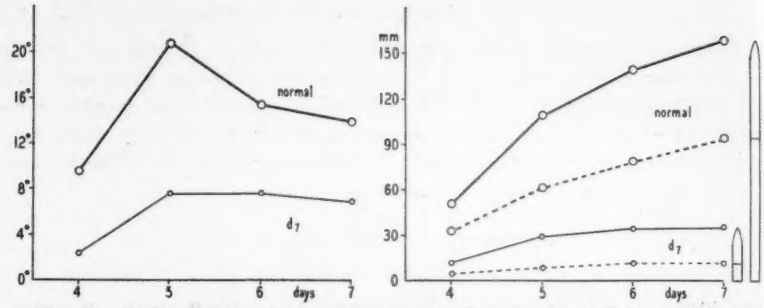

FIG. 7a. Left. Relation between the amount of auxin given off by the coleoptile and the age of the seedling in series segregating dwarf-7. Ordinate and abscissa as in figure 3a. Average values of 36 plants (60429, 60522, 60525).

FIG. 7b. Right. The length of dwarf-7 as compared with normal plants at various stages of development.

than by its short internodes. In the seedling stage the coleoptile is extremely small, whereas the length of the mesocotyl is of the type of *nana*. The small size of the leaves and the coleoptile may be correlated, since the coleoptile of grasses is considered to be a leaf (see **2**). The auxin production of pigmy plants is lower than in the normal ones. The type of curve resembles rather that of *nana* than those of the extreme dwarfs (fig. 8 a and b). The gene *py* is located in chromosome 6.

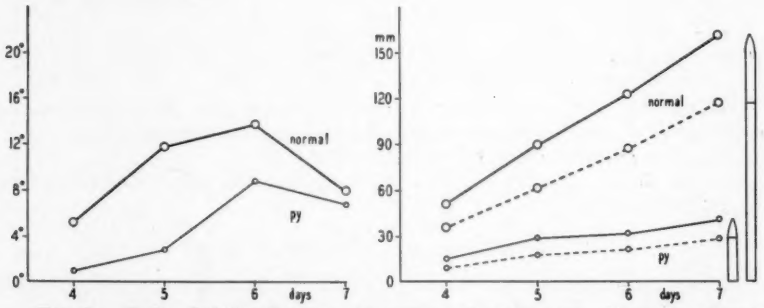

FIG. 8a. Left. Relation between the amount of auxin given off by the coleoptile and the age of the seedling in series segregating pigmy dwarfs. Ordinate and abscissa as in figure 3a. Averages of 36 plants (60513, 60512, 60427).

FIG. 8b. Right. The length of pigmy plants as compared with normal ones at various stages of development.

Summary

1. The amount of auxin given off by the coleoptile tips of etiolated maize seedlings was studied. Washing of the cut surface increases the amount of auxin diffusing out of these tips into agar blocks (table I).

2. The tips continue to give off auxin at approximately a constant rate for several hours after they are cut off (table II).

3. Isolated tips as short as 1 mm. continued to give off auxin for more than six hours at only slightly reduced rate at the end of that period (table III).

4. The production of auxin varies with the age of the seedlings. On the fifth to the sixth day after sowing, the production is optimal (fig. 1). At this stage about 2×10^{-10} gram of auxin per tip per hour is given off.

5. Seed producing *nana* dwarfs were found to contain the same amount of auxin as seed producing normal plants (fig. 2).

6. When 4 and 5 days old, *nana* dwarfs produce less auxin than do normal plants; when 6 and 7 days old, they produce equal amounts (fig. 3). The amount of auxin reaching the growing zones of the 6- and 7-day-old *nana* plants is much smaller, however, than in the normal plants (table IV). This is caused by the greater destruction of auxin in the *nana* dwarfs (table V).

7. The production of auxin in *dwarf-1, dwarf-2, dwarf-3, dwarf-7,* and the *pigmy* dwarf was found to be lower than that of their normal sibs (figs. 4 to 8).

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CORRELATION BETWEEN GROWTH OF EXCISED ROOT TIPS AND TYPES OF FOOD STORED IN THE SEED

GLADYS C. GALLIGAR

(WITH TWO FIGURES)

Introduction

Tissue culture had its inception near the close of last century (**2**), and during the past thirty-five years the cultivation of animal tissues has progressed markedly, while attempts to grow several types of plant tissue have met with varying degrees of success. The first reports on the growth of excised root tips were published by ROBBINS (**7**) and KOTTE (**4**) about twelve years ago. The object of the present investigation was to determine whether any correlation exists between the type of stored food in a given seed and the behavior of its excised root tip in sterile nutrient solution.

Materials and methods

Root tips from representative seeds containing high percentages of oil, sugar, starch, and protein, respectively, were selected to study their growth behavior when left undisturbed to grow to as great length as possible under the conditions imposed. Sunflower (*Helianthus annuus*), cotton (*Gossypium barbadense*), and castor bean (*Ricinus zanzibarensis*) were chosen for high oil content; Gradus wrinkled pea (*Pisum sativum*) and sweet corn (*Zea mays*) for high sugar content; Reid's Yellow Dent corn (*Zea mays*) and Hopi Indian corn (*Zea mays*) for starch; and Burpee's Extra Early smooth pea (*Pisum sativum*) and Manchu soybean (*Soja hispida*) for high reserves of protein.

The procedure to secure sterile root tips involved several steps and in the main followed that of ROBBINS (**7, 8**). The seeds were soaked for 4 hours in a solution of zonite diluted 1:30. They were then transferred by means of steel forceps to petri dishes containing a thin layer of 0.75 per cent. sterile agar, where they were allowed to germinate. The ends of the forceps were passed through a Bunsen flame and cooled in sterile water each time before touching the seeds. When the roots had grown approximately 1 inch, the tips were cut off in uniform lengths of 10 mm. by means of a steel scalpel, which was flamed and cooled before each operation. The excised root fragments were transferred with a sterilized wire loop to pyrex Erlenmeyer flasks of 125-ml. capacity carrying 50 ml. of sterile nutrient media. The flasks were kept in a laboratory with curtains drawn, where they received very weak diffuse light and where the temperature varied no more than 3° F. (68–71° F.) during the experiment.

The nutrient solution was a modification of Pfeffer's formula to which was added dextrose and peptone.[1] Immediately after being made, the solution was measured into the flasks, which were closed with cotton plugs and autoclaved at 15 lb. pressure for 20 minutes.

Germination was 90 to 100 per cent. Reid's Yellow Dent corn and the soybeans were secured from the Agriculture Experiment Station, University of Illinois. The Hopi corn was obtained from Indian traders in Arizona. All other seeds were ordered from the Burpee Seed Company.

From each species or variety 40 root tips were grown, with the exception of castor bean and soybean, from which sterile root tips were difficult to obtain. Five to ten individuals were grown in successive replicates, to avoid possible errors in growing only one series of 40 each. The cultures were maintained so long as growth was apparent as evidenced by noticeable elongation; some individuals, however, were left beyond this period with rather surprising results.

Attempts were made to measure increments of elongation at 12- and 24-hour intervals by means of a millimeter rule placed under the flask. The termination of the period of measuring was conditioned by the time required for the root tip to curve within the flask, which usually occurred on the tenth day with rapidly growing root tips.

The experiments were performed in the physiological laboratories at the University of Illinois.

Discussion of results

In every case except castor bean, table I shows that daily increments of length in individuals rise and decline sharply and irregularly with no evidence of a smooth sigmoid growth curve. This behavior may be caused by recovery from wound shock at the point of excision, or individual differences, or repeated adjustment to an artificial medium. The root tips were removed from their normal source of nutriment for growth and development to an artificial medium, from which they absorbed, perhaps rather poorly at best, the foods used in their metabolism. There can be no certain prediction of the behavior of protoplasm when it is subjected to a given set of external conditions. There is always the internal adjustment to the external environment, and this may be as variable as the number of individuals. Since the excised root tips used in this study behaved so differently and since no figures are available to demonstrate that a medium has been found with an optimal environmental complex perfectly adapted to the growth of isolated root tips of any variety or species, it is at present a great error to attach any practical significance to the results obtained. Until a medium is devel-

[1] $Ca(NO_3)_2$ 2.0 gm. KH_2PO_4 0.5 " KNO_3 0.5 " KCl 0.25 gm. $MgSO_4$ 0.5 " $FeCl_3$ 0.005 " Dextrose 2.0 per cent. Peptone 0.04 per cent. Distilled H_2O 6000 cc.

TABLE I

SAMPLE SERIES OF FIVE INDIVIDUALS OF EACH SPECIES SHOWING DAILY INCREMENTS OF LENGTH IN MILLIMETERS FOR EACH INDIVIDUAL DURING THE MEASURABLE STAGE

Individuals	Daily increment in millimeters															
	Sunflower															
1	2	3	3	0	9	6	5	3	0	2	2	3	...	...	...	
2	1	0	5	2	0	3	5	3	1	1	0	2	...	...	...	
3	0	0	8	2	4	5	6	1	0	0	0	0	...	...	...	
4	3	5	7	0	7	8	10	8	5	5	...	...	...	...	...	
5	3	0	4	1	0	2	3	1	1	1	1	1	...	...	...	
	Cotton															
1	3	3	3	5	2	0	2	2	0	0	1	...	...	...	...	
2	2	1	2	5	2	3	12	2	0	3	2	2	2	...	...	
3	2	2	1	3	3	0	0	0	0	1	1	0	0	...	...	
4	2	0	3	7	2	1	0	1	0	0	0	1	0	...	...	
5	2	0	0	1	1	2	3	4	0	0	1	...	...	...	...	
	Castor Bean															
1	5	0	0	1	1	0	0	0	0	0	0	0	...	...	...	
2	5	1	1	1	0	0	0	0	1	0	0	0	...	...	...	
3	5	2	1	0	0	0	0	0	0	0	0	0	...	...	...	
4	4	2	2	1	0	0	0	1	0	0	0	0	...	...	...	
5	5	2	1	1	1	0	0	0	0	0	0	0	...	...	...	
	Corn															
1	3	1	5	14	4	5	6	6	7	5	...	...	...	...	...	
2	13	7	0	3	5	10	3	2	5	5	...	...	...	...	...	
3	4	4	2	5	6	10	2	3	2	3	0	2	5	...	...	
4	3	2	2	3	7	3	2	4	4	3	3	0	5	...	...	
5	5	0	7	13	0	5	7	5	5	5	1	3	5	...	...	
	Hopi Indian corn															
1	7	3	3	5	5	5	5	5	3	2	2	3	4	4	2	
2	0	3	6	10	1	0	4	4	0	0	3	3	2	2	1	
3	1	2	4	4	5	0	13	5	4	3	1	3	3	1	2	
4	0	3	5	7	0	0	0	10	5	3	2	5	4	3	5	
5	1	1	1	0	2	0	3	3	0	2	1	0	2	2	2	
	Gradus pea															
1	2	1	2	2	2	3	0	0	0	0	2	0	0	...	...	
2	5	3	2	4	1	0	0	0	0	0	0	0	0	...	...	
3	2	1	2	1	1	2	0	0	0	0	2	0	0	...	...	
4	2	4	1	4	4	4	1	0	0	2	0	0	0	...	...	
5	3	2	2	3	3	3	1	0	0	0	0	0	0	...	...	
	Sweet corn															
1	2	3	3	0	9	7	5	3	0	2	2	3	...	...	...	
2	1	0	5	0	2	3	5	3	1	1	0	2	...	...	...	
3	0	0	8	2	4	5	6	1	0	0	0	0	...	...	...	
4	3	5	7	0	7	8	10	8	5	5	...	...	...	...	...	
5	3	0	4	0	1	2	3	1	1	1	1	1	...	...	...	

TABLE I (*Continued*)

Individuals	Daily increment in millimeters														
	Burpee's Extra Early Pea														
1	2	0	0	0	1	0	0	0	0	0	0	0	...	...	...
2	3	0	0	0	0	0	0	0	0	0	0	0	...	...	...
3	5	10	2	6	5	7	0	0	0	0	0	0	...	...	...
4	2	0	1	1	0	1	0	0	0	0	0	0	...	...	...
5	1	0	2	0	0	0	1	1	0	0	0	0	...	...	...
	Soybean														
1	0	0	0	0	0	0	0	0	0	0	0	0	...	...	...
2	0	0	0	0	0	0	0	0	0	0	0	0	...	...	...
3	1	1	0	1	0	0	0	0	0	0	0	0	...	...	...
4	1	0	1	1	0	0	0	0	0	0	0	0	...	...	...
5	2	1	1	1	0	0	0	0	0	0	0	0	...	...	...

oped in which the excised root tips respond similarly in their growth and development to their habits under natural conditions, it is hardly possible to work out any accurate usable knowledge in regard to their fundamental metabolism. Previous and present studies, however, are paving the way for subsequent work on this subject.

For a 10-day period the average daily rate of elongation of corn surpassed that of other root tips under observation (table II). Sweet corn and Hopi corn were approximately equal in their average daily growth and about three-fourths as rapid as dent corn. Next in order was cotton, rather closely followed by sunflower, both growing about one-half as rapidly as corn; Gradus pea exhibited a rate of about one-fourth, castor bean about one-fifth, and Burpee's Extra Early pea about one-eleventh the rate of dent corn. Root tips from soybean made little growth within the 10-day period. The greatest growth on the first day was made by an individual root tip of dent corn which increased 13 mm., a gain of 130 per cent. over the original length. The greatest increment achieved by any root tip on any day within the 10-day period was 14 mm., also made by dent corn. It is difficult to give accurate data concerning the rapidity of elongation after the measurable stage was passed, but certainly in some cases a marked growth was observed.

Dry weight determinations and final-length figures on the roots reveal some interesting contrasts to the daily rates of growth during the initial period (table II). Sunflower achieved an average length of 17.55 mm. in the 10-day period as compared to 23.6 mm. in cotton, but reached an average total length of 60.8 mm. as compared to 43 mm. in cotton, an approximate ratio of 3:2. On the other hand, the ratio of dry weights of the two is approximately 10:1, showing that cotton is less able to adapt itself to the nutrient medium, and in the process of inanition drew on more of its own stored reserves than did sunflower under the same conditions. Castor bean

TABLE II

SUMMARY OF DATA FOR 360 INDIVIDUAL ROOT TIPS CUT OFF AT ORIGINAL LENGTHS OF 10 MM. AND GROWN AS LONG AS POSSIBLE IN THE DARK IN MODIFIED PFEFFER'S SOLUTION PLUS 2 PER CENT. DEXTROSE AND 0.04 PER CENT. PEPTONE

SPECIES	AV. DAILY INCREASE IN LENGTH OF 30 ROOT TIPS FOR FIRST 10 DAYS										AV. TOTAL LENGTH AT END OF 10 DAYS	AV. FINAL TOTAL LENGTH	RANGE IN FINAL LENGTHS IN MM. AMONG INDIVIDUALS	FINAL AV. NO. LATERAL ROOTS	RANGE IN FINAL NO. LATERAL ROOTS AMONG INDIVIDUALS	AV. DRY WT. PER 10 ROOTS
	1	2	3	4	5	6	7	8	9	10						
Oily seeds	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*			*gm.*
Castor bean	4.71	1.20	1.05	0.70	0.24	0.00	0.00	0.25	0.23	0.00	8.39	16.2	11–23	0.0	0	0.031
Sunflower	2.65	1.55	0.60	3.70	2.65	3.04	1.63	0.52	0.97	0.31	17.72	60.8	23–175	27.0	0–63	0.244
Cotton	2.71	2.94	3.23	4.20	2.93	1.74	4.20	0.91	0.63	0.33	23.82	43.0	17–75	14.0	0–28	0.025
Starchy seeds																
Corn	4.81	3.15	5.70	3.92	4.35	5.00	4.21	3.27	4.89	5.77	45.07	241.4	36–1570	126.0	0–721	0.266
Hopi Indian Corn	1.82	2.41	3.80	5.21	2.67	1.15	7.05	4.42	2.01	1.80	32.32	103.7	23–430	21.0	0–45	0.055
Seeds containing sugar																
Gradus pea	1.86	1.95	2.20	1.65	1.67	1.64	0.73	0.62	0.32	0.25	12.89	20.2	10–53	2.0	0–8	0.034
Sweet corn	1.82	1.67	5.43	0.42	4.67	5.03	5.88	3.21	1.41	1.82	31.36	98.1	43–315	17.0	5–52	0.144
Seeds high in protein																
Burpee's Extra Early pea	1.75	1.00	0.72	0.65	0.55	0.65	0.30	0.35	0.20	0.00	6.17	22.9	10–47	0.12	0–1	0.029
Soybean	0.35	0.41	0.33	0.00	0.24	0.00	0.00	0.00	0.00	0.00	1.33	14.7	10–25	3.0	0–10	0.022

ceased activity and died too early for much of its own reserve to be drawn upon. It is quite obvious from the data at hand on castor bean, sunflower, and cotton that no correlation exists between oil reserves in seeds and subsequent growth behavior of excised root tips from such seeds under the conditions imposed.

Although dent corn manifested a much greater final elongation than any species used, the average dry weight was only slightly greater than that of sunflower. This indicates a greater ability on the part of the protoplasm of corn to prolong its activity in inanition by transfer of reserves in mature tissues to the actively growing meristem. It is possible, however, that the corn roots were unable to absorb as much nutriment from the medium as sunflower, and were thereby forced to draw more heavily upon their own reserves. Hopi corn and sweet corn also demonstrated an ability to maintain themselves by transfer of nutriment from their own mature tissues.

Gradus pea, Burpee's Extra Early pea, and soybean died before much of the stored reserves in the older portions of the root had been transferred to the growing tips. It is of interest to note that all of the root tips belonging to the family Leguminosae behaved poorly in comparison with the other root tips used.

In the progress of development under the conditions stated each species and variety exhibited certain peculiarities of its own, which will be described briefly in the following paragraphs.

Castor bean exhibited greatest increase in length on the first day, the rate gradually falling off until the fourth or fifth day, and showing no further increase in length except for an occasional millimeter or two on the sixth or seventh day. This species was the only one which exhibited anything like a fairly uniform growth rate. The root tips never developed laterals and never surpassed a total length of 25 mm., a gain of only 150 per cent over the original length. A grayish discoloration began on the third day, and covered the entire root by the time growth ceased.

The root tips of cotton invariably began to darken within 3 to 5 days, and, after growth stopped, turned completely black. From the ninth day onward lateral roots made their appearance, but never grew more than 13 mm. and never branched (fig. 1).

Sunflower root tips exhibited some very interesting responses. Secondary roots practically always appeared on the fifth day. Often, however, instead of producing secondary roots, there developed a series of cone-like papillae, which were approximately 2 mm. in diameter at the base and projected about 1.5 mm. from the surface of the main root (fig. 1). These papillae remained papillae in many instances; in other instances secondary roots pushed through the tops of these cones within 3 to 27 days after the formation of the papillae. Examination indicated that these elevations were

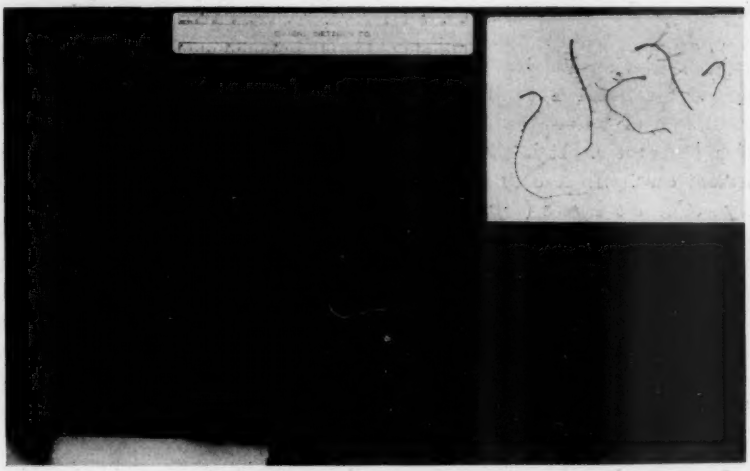

FIG. 1. Growth habits of several varieties and species of roots cut off when 10 mm. long and then allowed to grow without disturbance in Pfeffer's solution plus glucose and peptone. Left: four sunflower roots after four and one-half months' growth; the three short roots at upper left did not grow after the first month; note peculiar papillae and lateral roots developed from papillae. Upper center: a sunflower root that remained as the three in the upper left corner for 3 weeks; then produced lateral moniliform roots. Lower center: root of Hopi Indian corn, 4 months old and 430 mm. long. Upper right: cotton root tips five weeks old.

formed by secondary roots pushing against the outer two or three layers of cortical cells and the epidermis of the main axis, which did not yield to the growing tip of the secondary root for days or weeks and sometimes not at all. In the instances where the secondaries eventually emerged, they were exceedingly fine and threadlike and the papillae formed large collars around their bases. Occasionally the main axis of the root ceased elongation at 20 to 27 mm. with no secondaries growing through the characteristic papillae. On some of these individuals after a lapse of as much as two and one-half weeks, in some instances, laterals developed within 2 mm. of the tip and grew to lengths of 3 to 5 times those of the main axes with several branches of their own. This illustrates excellently that when growth in length ceases, differentiation proceeds close to the tip of the root. Many such laterals were very fine and threadlike; others of greater diameter possessed alternating regions of swelling and constriction, giving the appearance of a strand of spindle-shaped beads (fig. 1). Such moniliform roots are not unlike the peculiar constrictions secured in root tips of wheat cut at 0.1 mm. and grown in hanging drops by WHITE (9), who suggested that the constric-

tions were due to a "hyperhydric" change, a term first used by KÜSTER (5). HOTTES (3) has produced this peculiar beaded effect by dipping the tip of a growing root into cold water at daily intervals. When moniliform roots occur under natural conditions, they usually may be explained by fluctuation of water supply or by physical constriction by such agents as wire, stones, etc. It is obvious that none of the above explanations fit the present case. Since only sunflower root tips exhibited the phenomenon to any noticeable degree (figs. 1, 2), it appears to be a peculiar response of the protoplasmic activities of this species to the medium in which it was grown. Whenever a root of sunflower, as well as other roots, grew to such an extent that several of the growing points were pushed above the surface of the medium, root hairs formed in much greater abundance on the exposed parts, and were evident to within 1 mm. of the tip (fig. 1). It is common knowledge that ordinarily root hairs form less abundantly in water than in soil (1). The root tips of sunflower were translucent in the actively growing stage and turned brown with age.

Root tips of dent corn manifested a variety of forms in their growth, which was probably due to genetic differences, since no attempt was made to secure pure lines. The greatest growth among all the root tips was found in corn. In cases of excessive elongation fairly short and mostly unbranched secondary roots were formed. One individual root tip within a period of four and one-half months attained the astounding length of 1570 mm. with more than a hundred laterals (fig. 2). When such roots approached the end of their ability to continue elongation, lateral roots ceased forming with the result that several centimeters of the distal end were bare of secondaries (fig. 2). Occasionally, near the time when elongation ceased, there was a distinct thickening in the last 1 or 2 cm. of the tip. In cases where lateral roots developed profusely, the main axis failed to exhibit any great amount of elongation, a phenomenon which ROBBINS also observed. This behavior seems to support the view that the tip of the main axis, if vigorously growing, exercises an inhibiting influence upon the growth of the lateral branches of the axis. In general, lateral roots first appeared in corn no earlier than the seventh day and as late as the sixteenth day, with a few individuals producing no laterals at all. Such roots were thickened more than those possessing secondaries. Occasionally the cortex split away from the central cylinder near the proximal ends of the thickened roots, and also in a few of the roots with branches. Upon examination, the cortex of such roots was found to consist of huge cells elongated at right angles to the central cylinder. The interpretation is that the cortical parenchyma, when stimulated to hypertrophy, burst the bounds of the limiting epidermis, and in the resultant expansion split away from the central axis. This elongation and abnormal increase in size in the cells of the cortex was also observed in the

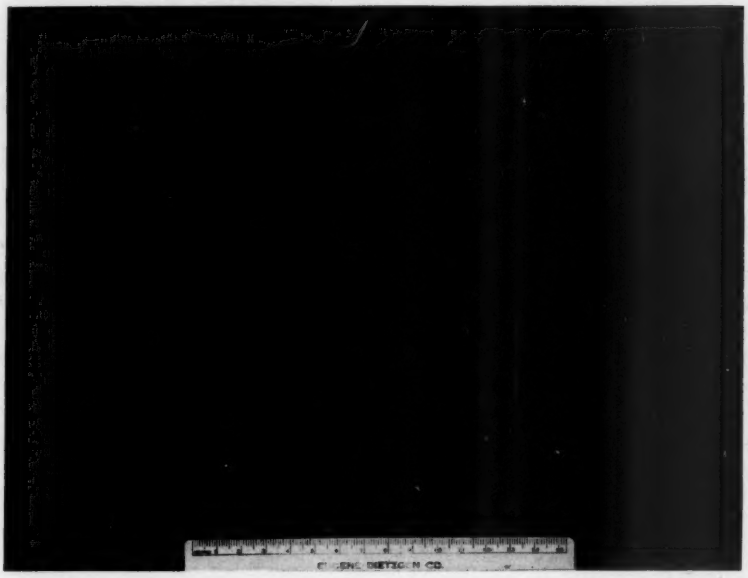

FIG. 2. Root of Reid's Yellow Dent corn, cut off when 10 mm. long, after four months of undisturbed growth in unchanged Pfeffer's solution plus glucose and peptone. This root, before drying, was 1570 mm. long and had 721 lateral roots.

swollen tips of the roots. In a few individuals, each lateral root was no more than 15 mm. long with the tip of each distinctly swollen. The cause of this interesting phenomenon is unknown, but as soon as the tips began to swell there was no further growth in length.

Hopi and sweet corn (fig. 1) behaved in a manner similar to that of dent corn with few exceptions. Neither attained lengths equal to those of dent corn; neither developed the characteristic swollen tips of dent corn; and the splitting of the cortex from the central cylinder was present to a lesser extent in both.

The root tips of Gradus pea grew slowly. After 7 to 10 days a pink coloration developed. When laterals appeared, they were always short, never exceeding 3 mm., and greatly thickened. In many cases these roots presented a corrugated appearance on their surfaces with the corrugations at right angles to the long axis of the root, resembling the contractile roots described by RIMBACH (**6**). Both cortex and epidermis grew faster than the central cylinder with the resultant wrinkling of the surface. This differs from the behavior of the dent corn roots, in which only the cortical cells

hypertrophied. There were some large cells in the cortex of the pea roots, but excessive cell division seemed also to have occurred.

With a few exceptions Burpee's Extra Early pea behaved in a manner similar to that of Gradus pea. They never elongated so much, were more slender, and developed secondary roots in only one instance.

Root tips of soybean after ceasing growth in the first week lay apparently inactive for 3 to 5 weeks, at which time a few hair-like secondaries appeared near the tip and in some instances grew 13 mm.

The uniformly abundant growth from the three varieties of corn suggested a short investigation to determine whether excised root tips from other moncots behaved equally well under the same conditions. Ten root tips each of wheat and onion in two series of five each were left to grow as near to maturity as possible. Onion was quite similar to Gradus pea except that the roots were more slender and developed no pigmentation. Wheat grew no more than 35 cm. and produced few secondaries. This seems to indicate that phyletic origin has no connection with the growth behavior of excised root tips.

Summary

1. There was some correlation between growth behavior of excised root tips and the type of food stored in seeds.

2. All root tips from seeds or grains high in starch reserves were able to grow well.

3. The growth behavior of root tips from seeds selected for oil and sugar varied widely.

4. Root tips from seeds with high protein reserves were least able to maintain growth.

5. There were no diurnal rhythms of growth.

6. There was no correlation between growth behavior and phyletic origin of root tips.

7. Root tips from Reid's Yellow Dent corn, Hopi corn, sweet corn, and sunflower were able to maintain themselves in active growing condition for a longer time, and achieved a greater total elongation and final dry weight than those of any other species or variety studied.

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INTERMEDIATES OF VITAMIN B_1 AND THE GROWTH OF *TORULA*

WILLIAM J. ROBBINS AND FREDERICK KAVANAGH

(WITH SIX FIGURES)

In previous papers (**2, 3, 4**) from this laboratory the effect of vitamin B_1 or its intermediates on the growth of excised tomato roots and of *Phycomyces blakesleeanus* Burgeff has been reported. Excised tomato roots (**2, 3**) required for unlimited growth either vitamin B_1 or thiazole[1] in addition to mineral salts and cane sugar. Pyrimidine alone did not permit growth to occur. *Phycomyces blakesleeanus* (**4**), on the other hand, did not grow (**3**) unless supplied with vitamin B_1 or with both intermediates. We have been interested in determining whether there are organisms which require pyrimidine but not thiazole. SCHOPFER (**5**) has reported that *Rhodotorula flava* and *Rhodotorula rubra* are such organisms. We have examined eight species of *Torula* and have found some which require an external supply of both intermediates for good growth under the conditions of our experiments, some which require pyrimidine but not thiazole and some which require neither.

Experimentation

A medium of the following composition was prepared:

KH_2PO_4	1.0	gm.
$MgSO_4 \cdot 7H_2O$	0.5	gm.
NH_4NO_3	0.05	gm.
Asparagine	0.5	gm.
Mineral supplements	0.1	cc.[2]
Dextrose (Cerelose)	50	gm.
Redistilled water	1000	cc.

The hydrion concentration of this solution was adjusted to pH 5.5 by the addition of Na_2HPO_4. Twenty-five cc. of the above solution were placed in 125-cc. Erlenmeyer flasks of pyrex glass. The solutions were divided into four groups. No addition was made to those of group I.

[1] Where thiazole is referred to in this paper the 4-methyl-5-β-hydroxyethylthiazole is meant and where pyrimidine is mentioned we mean the 2-methyl-5-ethyoxymethyl-6-aminopyrimidine. These compounds were used by WILLIAMS and CLINE (7) in synthesizing vitamin B_1 and are the intermediates in the formation of this vitamin.

[2] The mineral supplements were contained in a modification of HOAGLAND'S A to Z mixture prepared by adding to 18 liters of redistilled water: LiCl, 0.5 gm.; $CuSO_4 \cdot 5\ H_2O$, 1.0 gm.; $FeSO_4$, 1.0 gm.; H_3BO_3, 11.0 gm.; $Al_2(SO_4)_3 \cdot 18\ H_2O$, 1.0 gm.; $SnCl_2 \cdot 2H_2O$, 0.5 gm.; $MnSO_4 \cdot 4\ H_2O$, 7.0 gm.; $NiCl_2 \cdot 6\ H_2O$, 1.0 gm.; $Co(NO_3)_2$, 1.0 gm.; $TiOSO_4$, 1.8 gm.; KI, 0.5 gm.; NaBr, 0.5 gm.

To each flask of group II we added 30 units[3] (4.3 γ) of 4-methyl-5-β-hydroxyethylthiazole, to each flask of group III 30 units (5.0 γ) of 2-methyl-5-ethyoxymethyl-6-aminopyrimidine, and to each flask of group IV, 30 units of the thiazole and 30 units of the pyrimidine.

The flasks were sterilized at 12 lb. pressure for 20 minutes, and inoculated in triplicate with one drop of a suspension of *Torula*.

The *Torula* suspension was prepared by adding a loopful of *Torula* to 50 cc. of sterile distilled water. In removing the loopful of *Torula* care was taken to avoid including any of the wort agar on which the stock cultures were grown. The cultures were incubated at from 20° to 25° C.

The following *Torulae* supplied through the courtesy of F. M. Clark, Department of Bacteriology, University of Illinois, were used: *Torula hansen*, #2500; *T. sphaerica* (Hammar), #2504; *T. cremoris* (Hammar), #2512; *T. rosea*, #2519; *T. fermentati*, #2539; *T. kefyr*, #2540; *T. sanguinea* (Schimon), #2546; *T. laurentii*, #2547. The relative development of these eight organisms after ten days is shown in table I.

TABLE I

Growth of various species of *Torula* in a medium of mineral salts, asparagine and dextrose with the addition of thiazole or pyrimidine as indicated

Organisms	No addition	Thiazole only	Pyrimidine only	Thiazole and pyrimidine
Torula hansen	++	++	++	++
Torula sphaerica	+	+	+	+
Torula cremoris	–	–	–	–
Torula rosea	–	–	++++	++++
Torula fermentati	+	+	+	++
Torula kefyr	–	–	–	–
Torula sanguinea	–	–	++++	++++
Torula laurentii	+	+	+	++++

–=little or no growth. +=light growth.
++=medium growth. +++=fairly heavy growth.
++++=very heavy growth.

The organisms did not respond alike. Two of them, *Torula cremoris* Hammar and *T. kefyr*, grew very little if at all in any of the solutions. Evidently none of the media used was suitable for the growth of these two species.[4] As a result no information on the significance of the thiazole or

[3] 1 unit is 10^{-9} mole of the substance in question.

[4] A mineral nutrient solution containing asparagine and dextrose which was supplemented with vitamin B_1 and nicotinic acid amide gave negative results with these two organisms. They grew satisfactorily on a medium of agar, mineral salts, asparagine, dextrose, vitamin B_1, and neopeptone.

of the pyrimidine in their development was secured. Two species, *T. hansen* and *T. sphaerica* Hammar, grew to the same extent in all four solutions as far as could be judged from the turbidity of the solutions. The growth of *T. hansen* was heavier than that of *T. sphaerica*. Either these two *Torulae* do not require thiazole or pyrimidine or they synthesize sufficient of these compounds from the constituents of the medium for maximum growth under the conditions used. On the basis of evidence presented later, we are inclined to believe the latter explanation to be the correct one.

The growth of *T. laurentii* (fig. 1) and of *T. fermentati* was markedly improved by the addition of the mixture of thiazole and pyrimidine but

FIG. 1. *T. laurentii* grown in 1, basic medium (mineral salts, asparagine, and dextrose); 2, basic medium plus thiazole; 3, basic medium plus pyrimidine; 4, basic medium plus thiazole and pyrimidine.

little affected by either alone. The effect was greater for *T. laurentii* than for *T. fermentati*. These two species resemble *Phycomyces blakesleeanus* in requiring an external supply of both thiazole and pyrimidine, though the latter organism makes no growth in the absence of thiazole and pyrimidine while the two species of *Torula* under discussion grew slightly in the solution which lacked both thiazole and pyrimidine. It is possible that the small amount of growth of these two *Torulae* in the solutions lacking thiazole and pyrimidine or containing one of the compounds alone was made at the expense of small amounts of vitamin B_1 or of its intermediates carried in or with the cells of the original inoculum. Subcultures of these organisms from the growth in the absence of the intermediates to a solution of similar composition were not made. It would seem justifiable, however, to describe *T. laurentii* and *T. fermentati* as organisms requiring an external supply of pyrimidine and thiazole for good growth in the medium we have used. They may synthesize small amounts of the two intermediates, but if so the quantity formed under the conditions of our experiments was not sufficient for maximum growth.

T. rosea (fig. 2) and *T. sanguinea* grew very poorly in the solutions which lacked thiazole and pyrimidine and in those to which thiazole alone was added. They developed heavy growth in the solutions to which pyrim-

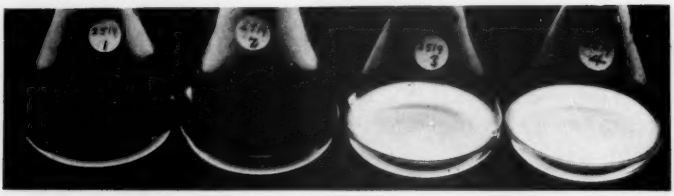

FIG. 2. *T. rosea* grown in 1, basic medium (mineral salts, asparagine, and dextrose); 2, plus thiazole; 3, plus pyrimidine; 4, plus thiazole and pyrimidine.

idine was added and in the solutions to which thiazole and pyrimidine had been added. Two possible explanations for these results may be offered. *T. rosea* and *T. sanguinea* may not synthesize either thiazole or pyrimidine and require only pyrimidine for normal growth, or they require both and synthesize the thiazole only. We are inclined to believe that the latter explanation is correct.

In order to substantiate in part the assumptions made on the ability of these various species of *Torula* to synthesize thiazole or pyrimidine, cultures of representatives of six species were sterilized at 12 lb. pressure for 10 minutes after 15 days' growth in the media described above. The sterile solutions containing the *Torula* cells and products of their metabolism

TABLE II

GROWTH OF *Phycomyces blakesleeanus* IN A MEDIUM OF MINERAL SALTS, ASPARAGINE AND DEXTROSE AND ADDITIONS GIVEN ABOVE IN WHICH VARIOUS SPECIES OF *Torula* HAD GROWN. COMPARE WITH TABLE I. FIGURES GIVEN ARE THE pH OF THE SOLUTIONS WHEN INOCULATED WITH THE *Phycomyces*

ORGANISMS	NO ADDITION	THIAZOLE ONLY	PYRIMIDINE ONLY	THIAZOLE AND PYRIMIDINE
Torula hansen	+++	+++	+++	+++
	3.6	3.5	3.5	3.5
Torula sphaerica	–	+	–	+++
Torula fermentati	+	+	+	+++
Torula laurentii	–	–	–	+
	3.6			3.8
Torula rosea	–	–	++	+++
	4.3	4.2	3.8	3.8
Torula sanguinea	–	–	+++	+++
	3.7	3.7	3.6	3.9

+++ = Aerial mycelium 2 cm. high, sporangiophores and sporangia.
++ = Submersed mycelium with few scattered sporangiophores.
+ = Submersed mycelium covering bottom of flask; no aerial hyphae.
– = Little or no growth.

were inoculated with spores of *Phycomyces blakesleeanus*. This fungus requires both thiazole and pyrimidine for growth. Its development would therefore demonstrate the presence of both intermediates. Its failure to grow in a culture solution in which a species of *Torula* had grown might be the result of a deficiency of one or both of the intermediates or of the development of some harmful constituent in the solution.

The relative growth of *Phycomyces* in these solutions in which a species of *Torula* had grown is given in table II. Some determinations of the hydrion concentrations of the solutions after the growth of the *Torula* and before inoculation with *Phycomyces* are also given in this table.

Phycomyces blakesleeanus developed fairly well in all four of the solutions in which *T. hansen* had grown. Aerial mycelium about 2 cm. high with sporangiophores and sporangia formed in all the culture solutions (fig. 3). The growth of the *Phycomyces* was probably limited by the con-

FIG. 3. *Phycomyces blakesleeanus* grown in solutions in which *T. hansen* had grown. From left to right: 1, basic medium; 2, plus thiazole; 3, plus pyrimidine; 4, plus thiazole and pyrimidine.

centration of asparagine (0.05 per cent.) in the original medium. We may conclude that *T. hansen* synthesized both thiazole and pyrimidine (or vitamin B_1) from the constituents of the medium. From the growth of this *Torula* (see table I) in the four solutions we may conclude also that the amount of the thiazole and pyrimidine synthesized was adequate for maximum growth in the medium used. Otherwise some increase in growth

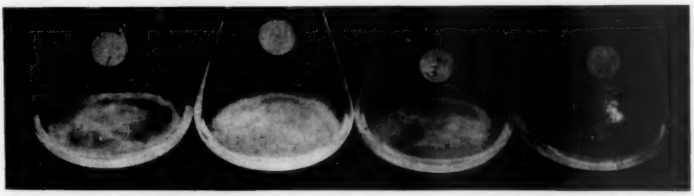

FIG. 4. *Phycomyces blakesleeanus* grown in solutions in which *T. fermentati* had grown. From left to right: 1, basic medium; 2, plus thiazole; 3, plus pyrimidine; 4, plus thiazole and pyrimidine.

would have occurred in the cultures to which thiazole and pyrimidine were added.

Phycomyces grew in all four of the solutions originally containing *T. fermentati.* The growth was greater in the solutions to which both thiazole and pyrimidine had been added (fig. 4). We conclude that *T. fermentati* synthesized both pyrimidine and thiazole but in smaller amounts per flask than *T. hansen* did. The amount of thiazole and pyrimidine synthesized was not sufficient for maximum growth of this *Torula* in the medium used (see table I) since the addition of the two intermediates improved its growth.

Phycomyces grew very little in the medium with no supplement or with pyrimidine in which *T. sphaerica* had grown but formed some submersed mycelium in the solution supplemented with thiazole (fig. 5). We conclude

FIG. 5. *Phycomyces blakesleeanus* grown in solutions in which *T. sphaerica* had grown. Left to right: 1, basic medium; 2, plus thiazole; 3, plus pyrimidine; 4, plus thiazole and pyrimidine.

that this *Torula* may have synthesized a small amount of pyrimidine but little or no thiazole. The failure of *Phycomyces* to develop more luxuriantly in these solutions would not appear to be the result of any injurious substances formed by the *Torula* since the growth of the *Phycomyces* was as great in the mixture of pyrimidine and thiazole as it was in the solutions in which *T. hansen* had grown. These results are not entirely clear when compared with the relative growth of *T. sphaerica* (see table I). If pyrim-

FIG. 6. *Phycomyces blakesleeanus* grown in solutions in which *T. rosea* had grown. Left to right: 1, basic medium; 2, plus thiazole; 3, plus pyrimidine; 4, plus thiazole and pyrimidine.

idine and thiazole are required for growth by this *Torula*, we should have expected greater development of *Phycomyces* in the medium containing no supplements and in the medium with pyrimidine.

Phycomyces made little or no growth in the solutions with no supplements and those with thiazole only in which *T. rosea* or *T. sanguinea* had been inoculated (fig. 6). The growth in the solutions containing pyrimidine was better. We conclude that these two *Torulae* synthesize little or no pyrimidine but form thiazole. The amount of thiazole per flask formed by the growth of *T. sanguinea* was somewhat greater than that formed by *T. rosea*. The relative growth of the two organisms in these solutions (table I) would suggest that the amount of thiazole formed by these two *Torulae* was adequate for their maximum growth in the medium used.

Phycomyces grew little or none in the solution with no addition, the thiazole solution, and the pyrimidine solution in which *T. laurentii* had grown. Its developument in the solution with both intermediates was poor. This suggests that this *Torula* had developed some material or materials which were injurious to *Phycomyces*. If this is correct our experiments would not permit us to draw conclusions on the formation of thiazole or pyrimidine by *T. laurentii*.

Discussion

These observations on the growth of eight *Torulae* in solutions lacking thiazole and pyrimidine and in solutions containing one or both of the intermediates, together with those on the subsequent growth of *Phycomyces blakesleeanus* in the same solutions, lend support to the hypothesis that vitamin B_1 is a fundamental growth factor. Some organisms make enough for adequate growth, others do not. Of those which do not, some synthesize neither the pyrimidine nor the thiazole in adequate quantities, others synthesize thiazole and some are capable of synthesizing pyrimidine. The result is that there are organisms which must be supplied with vitamin B_1 or both intermediates for satisfactory growth (*Phycomyces blakesleeanus, Torula laurentii, Torula fermentati*). There are organisms which must be supplied with pyrimidine or vitamin B_1 (*T. rosea, T. sanguinea*), and still others which must be supplied with thiazole or vitamin B_1 (tomato root).

It is possible to assume that the thiazole and the pyrimidine function in the organism as such, and that when vitamin B_1 is an effective growth substance it is split into its intermediates which then play their rôle in metabolism. As an alternate hypothesis we may assume that the vitamin molecule as such is essential and that the pyrimidine and thiazole synthesized by the organism or furnished from without are used to form the vitamin B_1 molecule. SCHOPFER favors the first of these possibilities. We

have presented evidence elsewhere supporting the second hypothesis. The demonstration that *Torulae* which grew in a solution lacking B_1 or its intermediates synthesized both intermediates supports our assumption. It is supported also by the demonstration that a *Torula* which grew in a medium supplemented with pyrimidine only synthesized thiazole.

OKUNUKI (**1**) noted the favorable action of red yeasts on the development of certain fungi. WASSINK (**6**) observed good development of *Phycomyces blakesleeanus* in a maltose medium which had become contaminated with a red yeast. Without the contamination little or no growth of the fungus occurred in the medium. These results may probably be explained by the formation of vitamin B_1 or of thiazole and pyrimidine by the yeasts concerned.

Summary

Eight species of *Torula* were grown in a medium of mineral salts, asparagine, and dextrose, and the same medium to which the thiazole alone, the pyrimidine alone, or both intermediates of vitamin B_1 were added. Two species failed to grow in any of the four media; the growth of two was unaffected by the supplements; two grew distinctly better when both intermediates were used as supplements but were unaffected by either intermediate alone; the growth of two was much increased by the addition of pyrimidine or of pyrimidine and thiazole but unaffected by thiazole alone. By cultivating *Phycomyces blakesleeanus* in the solutions in which various species of *Torula* had grown it was demonstrated that a *Torula* which grew in the basic solution had synthesized thiazole and pyrimidine and one which grew in the solution supplemented with pyrimidine alone had synthesized thiazole.

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RELATIONSHIP OF THE ORGANIC ACIDS OF TOBACCO TO THE INORGANIC BASIC CONSTITUENTS[1]

GEORGE W. PUCHER, HUBERT BRADFORD VICKERY, AND ALFRED J. WAKEMAN

Introduction

There is little doubt that one of the important functions of the organic acids commonly found in relatively substantial proportions in leaf tissues is to provide a means whereby the hydrogen-ion activity of the solution in the cells is maintained within the proper limits for the particular species. There is considerable literature on the buffer capacity of plant juices (**3, 4, 5**), and it is obvious that the combined effects of the partially neutralized organic acids, and of the phosphoric acid, as well as of the amino acids, and posssibly, to some extent, of the soluble proteins result in systems that are remarkably stable with respect to change in hydrogen-ion activity.

Owing to the inadequacies of the methods available until recently, few reliable studies have been made of the detailed organic acid composition of leaves, and for determinations of total acidity many investigators have depended entirely upon titration of water extracts of the tissues. Such data furnish a measure merely of that part of the water-soluble acids that remains unneutralized at the reaction of the cells and give little indication of the total quantity present either as soluble or insoluble salts. In order to ascertain the true organic acidity it is necessary to liberate the acids from combination and isolate them as a group; the total quantity present can then be determined by titration between properly selected pH limits. With this information available it becomes possible to investigate the relationships between the chief acid- and base-binding substances of the tissue.

Data obtained in the course of a study of these factors in tobacco leaf tissue are described in the present communication. We were fortunate in having at our disposal a series of samples derived from plants that had been grown with especially careful control of the inorganic constituents of the fertilizer applied. Complete analyses of the ash of these samples had been made by Dr. E. M. BAILEY of the Connecticut Agricultural Experiment Station and published in connection with BAILEY and ANDERSON'S studies (**1**) of the effect of various soil treatments on the technical quality of the leaf. We are indebted to them for permission to carry out organic acid analyses of these samples and to recalculate and quote their data.

[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington.

Experimentation

The samples used in the experiments consisted of leaves that had been subjected to the processes of curing and of fermentation. It was therefore desirable first to establish the nature of the effect of these operations on the organic acids. Samples of tobacco leaves picked the same day were allowed to cure for various periods in the customary way. Each sample consisted of 60 leaves of approximately the same total initial weight. The results of the analyses were calculated upon an arbitrary basis of 1 kilo of original fresh weight of each individual sample. The data referring to the nitrogenous constituents and the carbohydrates have been discussed in a previous publication (**7**). The results of the determinations of the total organic acidity are given in table I.

TABLE I

TOTAL ORGANIC ACIDITY OF TOBACCO LEAVES AT VARIOUS STAGES IN THE CURING PROCESS

HOURS	ACIDITY*
	meq./kgm.
0	320
41	367
64	340
87	285
111	320
159	272
185	275
207	347
231	357
279	308
303	339
Average	320.9 ± 22.1

* Data are expressed in milliequivalents per kilogram of original fresh weight of each sample.

These figures show that little or no change occurs in the total organic acidity throughout the period of curing. The probable error is ± 6.9 per cent., which is the order of precision to be expected for measurements of this type. Alterations in the relative proportions of individual acids may, and in fact do, occur, but there is no substantial conversion of organic acids into nonacidic metabolic products, nor is there evidence for the accumulation of acid end products of the metabolism of other substances.

The information available on the effect of fermentation on the organic acids is very limited. HALEY, NASSET, and OLSON (**2**) carried out determinations of the ether-soluble organic acidity of parallel samples of cured and fermented tobacco, employing a method not unlike that used in this

laboratory. The average of nine determinations made on web tissue before and after fermentation differed by only 3 per cent., and it seems fair to assume therefore that such changes as may occur during fermentation are of a minor nature. This conclusion is supported by inferences drawn from the reaction of the tissues. The reaction of water extracts of our fermented samples ranged from pH 6 to 6.5—values of exactly the same order of magnitude as those of extracts from many samples of cured tissue. Important alterations in the proportion of organic acids present would have changed this reaction appreciably inasmuch as the inorganic basic constituents did not change. For the present purposes, however, comparisons are to be made between samples, all of which had been subjected to fermentation in the same way; minor changes that may have taken place in the organic acids accordingly have no significance.

The samples were derived from two different crops, 1924 and 1927, respectively a dry and a rainy season, and represented a considerable variety of cultural treatments. The two crops differed widely in the technical quality of "fire-holding capacity," the 1927 crop being classified as good, the 1924 crop as poor in this relation. The soil treatments of each individual plot were the same in each year, and had been maintained the same for the crops grown during the intervening years on the same plots. All samples were graded by experts, and the so-called darks, "D," and seconds, "S" were employed for the present work. These two grades represent leaves derived respectively from the upper and from the lower parts of the plant.

The detailed analyses of the ash and of the intact tissue were recalculated for the present purpose in terms of milliequivalents per 100 gm. of dry leaf. The positive ions calcium, magnesium, potassium, nicotine, and ammonium, and the negative ions phosphate, chloride, sulphate, and nitrate, were employed. Phosphorus and nicotine were reckoned as monovalent ions in the calculations because the tissue reactions were close to pH 6. The analytical figures for sulphate reported by BAILEY and ANDERSON represent both organic and inorganic sulphur, but a study of the sulphur distribution of the samples showed that approximately 70 per cent. was in the form of inorganic sulphate; much of the balance doubtless represents sulphur combined in proteins. The factor 0.7 was therefore used to correct the total sulphur determinations of BAILEY and ANDERSON in order to arrive at an approximation to the quantity of inorganic sulphate. An analogous correction was not applied to the phosphate data although the determinations represent total phosphorus in the ash, and a part of this may have originated from organic phosphorus compounds. It is assumed that all of the phosphorus represents monovalent phosphoric acid radicals as there is little doubt from the nature of the known organic phosphorus compounds of plant tissues that a part of the phosphorus even in such compounds fulfills

this function. In any case, the error involved in this assumption has little effect upon the conclusions to be drawn as phosphoric acid forms so small a part of the total inorganic acidity present. The nature of the compounds of silicon in the tissues presents a very puzzling problem, and it would appear from the present data that very little if any of the silicon dioxide determined in the ash represents silicic acid or analogous acidic substances.

The sum of the positive ions present in the ash, together with the ammonia and nicotine, may be taken to represent the total acid-binding capacity of the tissues. The sum of the inorganic negative ions represents the base-binding capacity of the tissues due to inorganic acids. In all cases there is a large excess of positive ions, and it is the purpose of the present paper to show that this excess is closely related to the quantity of ether-soluble organic acids in the intact tissue.

It should, perhaps, be emphasized that the comparison is made between the excess of positive ions present at approximately the tissue reaction of pH 6 and the *whole* of the ether-soluble organic acids that can be titrated between the limits pH 7.8 and 2.6. This involves the assumption that the proportion of organic acids in combination with bases is substantially fixed, notwithstanding possible variation from sample to sample in the chemical composition of this group of substances. Furthermore, in the calculation of the bases it is assumed that the inorganic constituents of the ash are all present in the intact tissue in ionic form capable of salt formation. This is certainly not entirely true; for example, a part of the magnesium is involved in the fresh tissues in complex combination in the form of chlorophyll. The fate of this part of the magnesium after the technical processes of curing and fermentation have been carried out is entirely unknown.

The data assembled in table II permit a comparison between the excess acid-binding capacity of the individual samples from the 1927 crop and the total organic acidity as determined by the method of PUCHER, VICKERY, and WAKEMAN (**6**) after ether extraction.

The degree with which one variable depends on the variation of another is probably most easily appreciated from a consideration of the correlation coefficient calculated in the customary way. In the present case we have two sets of data, one obtained from plants fertilized with various potassium compounds, the other with lime. Each set of 12 samples contains 6 lots of leaves graded as "darks" and 6 graded as "seconds." These grades imply a difference in position on the plant as well as intangible elements of technical "quality," but for the present purpose this may be neglected and the two series grouped together.

The correlation coefficient between the excess positive ions and the total organic acidity for the 12 samples from plants grown on the potash plots is 0.833 ± 0.060; that for the other set is 0.905 ± 0.035. A similar calcula-

tion for the entire set of data from the 1924 crop (13 samples) gave a correlation coefficient of 0.812 ± 0.067. These coefficients indicate that the quantities are closely dependent on each other.[2]

Although the statistical analysis of the data leads to the conclusion that the two variables are closely interdependent, it in no way suggests which is the independent and which the dependent variable. It is not possible to conclude that certain plants absorbed more basic ions from the soil and therefore elaborated more organic acids. It may be equally true that certain plants manufactured more organic acids and therefore absorbed more inorganic base from the soil. Some entirely different factor may indeed have been the true independent variable, and it is quite possible that the mutually related organic acidity and excess basic ion content varied in response to the changes in this other factor.

Inspection of the data of table II shows that the inorganic acidic components of the tissue (negative ions) play a minor rôle in maintaining the balance between the positive and negative ions of the tissue. The average of the total inorganic anions amounts to only about one-sixth of the average total cations in the samples from the potash plots and to one-tenth for the samples fertilized with lime. Organic acidic substances, particularly in the latter case, obviously dominate the situation.

It must be emphasized that we are in a position to deal from the analytical point of view only with that part of the organic acids of the tissues that can be extracted with ether under suitable conditions. No accurate estimate can at present be made of base-binding substances that cannot be extracted with this solvent. The tobacco leaf contains considerable quantities of pectins and allied compounds, some of which possess base-binding properties. If the assumptions upon which the present calculations have been founded are correct, it would appear that roughly one-third of the excess positive ions are, on the average, combined with acidic substances other than ether-soluble organic acids.

The acids actually determined by the method employed are chiefly malic, oxalic, and citric; these three make up about four-fifths of the ether-soluble acidity. The exact chemical nature of this group is, however, immaterial to the present discussion.

If it be accepted that the high correlation between the excess positive ions and ether-soluble organic acidity in tobacco leaf tissues demonstrates a mutual interdependence between these quantities, inferences may be drawn from average values and extended to the consideration of other sets of

[2] The high significance of these coefficients may be appreciated from comparison with FISHER'S table (SNEDECOR, G. W. Statistical methods. Ames, 1937, p. 125). For 10 degrees of freedom the coefficient at the 1 per cent. level of significance is 0.708, for 11 degrees of freedom it is 0.684.

TABLE II

RELATIONSHIP BETWEEN POSITIVE AND NEGATIVE IONS OF ASH, TOGETHER WITH AMMONIA AND NICOTINE, AND TOTAL ORGANIC ACIDITY OF INTACT TISSUE IN SAMPLES OF FERMENTED TOBACCO LEAF FROM CROP OF 1927

POTASH SERIES*						LIME SERIES*					
SAMPLE NO.	GRADE	TOTAL POSITIVE IONS	TOTAL NEGATIVE IONS	EXCESS POSITIVE IONS	TOTAL ORGANIC ACIDITY	SAMPLE NO.	GRADE	TOTAL POSITIVE IONS	TOTAL NEGATIVE IONS	EXCESS POSITIVE IONS	TOTAL ORGANIC ACIDITY
608	D	385.8	81.4	304	175	620	D	430.4	34.9	396	245
610	D	402.0	65.2	337	207	622	D	396.4	65.9	331	223
612	D	385.7	64.3	321	177	624	D	413.1	39.8	373	272
614	D	403.7	63.7	340	176	626	D	388.3	44.4	344	216
616	D	430.4	84.9	346	205	628	D	450.8	27.7	423	304
618	D	407.4	70.8	337	206	630	D	388.3	40.1	348	212
Average		402.6	71.7	331	191			411.2	42.1	369	245
609	S	443.0	98.0	345	183	621	S	470.2	33.3	437	302
611	S	445.7	87.7	358	250	623	S	411.1	50.5	361	250
613	S	424.6	73.6	351	219	625	S	444.8	29.0	416	290
615	S	418.1	57.9	360	213	627	S	383.7	47.6	336	253
617	S	434.6	69.6	365	262	629	S	488.3	27.0	461	335
619	S	435.7	53.3	382	211	631	S	404.1	23.7	380	272
Average		433.6	73.3	360	223			433.7	35.2	398	284
		Correlation coefficient $r = 0.833 \pm 0.060$						Correlation coefficient $r = 0.905 \pm 0.035$			

* Figures are milliequivalents per 100 gm. of dry tissue.

samples. It may be useful to present the data from this point of view. In table III are shown the average values of the organic acidity and of the excess positive ions of samples of grade D from two crops fertilized each in two ways. In the upper portion of the table are details from which the relative order of magnitude of the individual bases and of the inorganic acidity may be appreciated. Calcium and potassium clearly occupy the dominant position among the bases.

The organic acidity of the samples grown on the potash plots in 1924 was distinctly higher than that of the samples grown in 1927. In the later crop, there was a marked decrease in total positive ions, partly due to a diminution in calcium content, and there was a corresponding decrease in inorganic acidic ions. The compensation was not, however, complete so that the ratio between the excess positive ions and the organic acidity changed from 1.52 to 1.73.

The samples from the lime-fertilized plots showed a slight increase in organic acidity and in excess positive ions. There was a marked decrease in calcium and potassium in the 1927 crop which was only partially compensated by an increase in magnesium. The substantial drop in inorganic negative ions, however, completed the compensation and preserved the balance between the excess positive ions and the organic acids. The ratio did not change significantly.

If the potash series of 1924 is compared with the lime series of the same

TABLE III

RELATIONSHIP BETWEEN POSITIVE AND NEGATIVE IONS OF SAMPLES OF TOBACCO OF GRADE D DERIVED FROM THE CROPS OF 1924 AND 1927

	Potash series*		Lime series*	
	1924	1927	1924	1927
Calcium	180	148	200	161
Potassium	166	173	150	128
Magnesium	67.7	55.6	54.7	90
Nicotine	14.6	10.6	16.3	13.7
Ammonia	33.2	15.6	39.3	18.6
Total positive ions	462	403	460	411
Total negative ions (inorganic acidity)	107	71.7	110	42
Excess positive ions	355	331	350	369
Organic acidity	234	191	236	245
Ratio $\frac{\text{excess positive ions}}{\text{organic acidity}}$	1.52	1.73	1.48	1.51

* Figures are averages of from four to six individual samples and are expressed in milliequivalents per 100 gm. of dry tissue.

year, it is clear that there was no significant change in the organic acidity nor in the excess positive ions. The inorganic acidity is also practically the same, the slight difference in excess positive ions being attributable to minor differences in calcium and potassium. But if the potash series of 1927 is compared with the lime series of that year, there is an important increase in organic acidity in the samples from the lime plots. There is only a small increase in total positive ions, but there is a substantial diminution in the inorganic negative ions, so that the excess of positive ions increases materially. Nevertheless, the change falls a little short of being strictly proportional to the change in organic acids, the ratios being 1.73 and 1.51 respectively.

A similar comparison between the "D" samples and the "S" samples from the 1927 crop is shown in table IV. These grades to a considerable extent represent position of the leaves on the plant, the "S" grade being mainly bottom leaves, the "D" grade, upper leaves. The bottom leaves from the potash plots contain more organic acids than top leaves from plants fertilized in the same way. The inorganic negative ions are practically the same, and it is clear that the balance of ions is maintained by the organic acids. The same is true of the two sets of samples from the lime plots. Differences between the lime and potash samples within each group are mostly caused by the lower inorganic negative ions in the lime series with correspondingly higher organic acids.

TABLE IV

RELATIONSHIP BETWEEN POSITIVE AND NEGATIVE IONS OF SAMPLES OF GRADE D AND GRADE S FROM THE CROP OF 1927

	"D" SAMPLES* TOP LEAVES		"S" SAMPLES* BOTTOM LEAVES	
	POTASH SERIES	LIME SERIES	POTASH SERIES	LIME SERIES
Calcium	148	161	177	180
Potassium	173	128	181	120
Magnesium	55.6	90	62.7	112
Nicotine	10.6	13.7	5.7	9.9
Ammonia	15.6	18.6	7.4	11.5
Total positive ions	403	411	434	433
Total negative ions (inorganic acidity)	71.7	42	73.3	35.2
Excess positive ions	331	369	360	398
Organic acidity	191	245	223	284
Ratio $\frac{\text{excess positive ions}}{\text{organic acidity}}$	1.73	1.51	1.61	1.40

* Figures are averages of six samples and are expressed in milliequivalents per 100 gm. of dry tissue.

It is apparent that the ether-soluble organic acidity of tobacco leaf tissue may vary between relatively wide limits, and that this variation is associated with the manner in which the plants have been fertilized. The data indicate with some clearness that the fluctuations are in the direction which, from the chemical composition of the tissue, may be expected to maintain an equilibrium between the positive and negative ions such that the hydrogen-ion activity of the tissues does not vary in any significant manner. Many factors undoubtedly come into play, but the nature of the inorganic ions that are assimilated and the total amount present have a direct influence. The organic acids are therefore definitely concerned in the problem of inorganic nutrition, and a comprehensive study of the manner in which these substances may vary in response to the cultural treatment of the plant is much to be desired.

Summary

1. If it be assumed that the major constituents of the ash of cured and fermented tobacco leaves, with the exception of silicon dioxide, represent respectively basic or acidic ions combined essentially as salts in the cells, and if, in addition, allowance is made for the nicotine, ammonium, and nitric acid of the tissues, it is possible to calculate the total positive ions and the total negative ions present in terms of chemical equivalents.

2. In all cases examined there is a large excess of positive ions and the quantity so found is closely correlated with the quantity of ether-soluble organic acids as determined by a suitable titration method.

3. The organic acids occupy a dominating position with respect to the balance of positive and negative ions in the tissues, and it is inferred that these substances are closely concerned in the phenomena of inorganic nutrition.

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GROWTH OF CABBAGE SEEDLINGS IN SAND CULTURE, AS AFFECTED BY DELAYED APPLICATION OF NUTRIENT SALTS

A. A. DUNLAP

(WITH TWO FIGURES)

Introduction

When some kinds of seeds are thickly planted, the resulting seedlings are likely to develop excessively elongated hypocotyls and to be otherwise unsuitable for transplanting. It has long been known that excessive elongation of many kinds of stems, including hypocotyls, is generally correlated with inadequate light intensity, as in partial or complete etiolation, when conditions of temperature and water supply are adequate and when inorganic nutrients are available. Without referring particularly to numerous earlier papers on the etiolation of ordinary plants, reference may be made to POPP's (**6**) observation that stem elongation in early growth stages of soy bean was approximately inversely proportional to the intensity of the light employed. Reference may be made also to the experiments of VAN OVERBEEK (**5**), who found that the rate of hypocotyl elongation and the final length attained by radish hypocotyls were only about half as great when the seedlings were grown under ordinary conditions as when they were grown in darkness. That different kinds of plants should show great differences in respect to this influence of light is only to be expected (MACDOUGAL's classic book on light effects, **4**), and the physiological problem thus raised is surely one of great complexity. In connection with the study here reported, the employment of artificial illumination in the greenhouse at night was only slightly effective in retarding and limiting hypocotyl elongation in cucumber seedlings.

In this study it has been found that under ordinary greenhouse conditions seedlings of cabbage, cucumber, and tomato generally become "drawn," showing some of the growth symptoms of etiolation, when the seeds are sown too close together, but exhibiting a quite satisfactory degree of stockiness when they are allowed to grow a few centimeters apart. Dense stands of perfectly satisfactory seedlings have been grown in an ordinary greenhouse, however, by employing sand as substrate, to which suitable nutrient salts had been added in suitable concentrations (**2**). Ammonium nitrate was found to be very satisfactory when initially added at the rate of about 0.5 gm. per liter of sand, the pH value of the solution used being between 5 and 7; the sand used contained a trace of available phosphorus. Some other ammonium salts gave similar results, but leaf injury was likely to follow their use unless

a potassium salt (such as KCl, K_2SO_4) was also added initially, at suitable concentration. In any event, this desired stunting effect of an NH_4^+ salt was secured only when the initial salt concentration was properly adjusted. With concentration too high, toxic effects were produced; with concentration too low, the crowded seedlings showed the usual condition of partial etiolation. Injury from the use of NH_4^+ salts was especially likely to occur when tall sand columns were employed with automatic tray irrigation (3).

The present paper reports some recent greenhouse experiments in which satisfactorily stocky seedlings were obtained in crowded stands without the use of NH_4^+. These were grown in pots of washed sand with tray subirrigation (3) and without addition of any nutrient salt to the sand until 10 days after the seedlings had emerged. At that time a delayed fertilizer treatment, with KNO_3 and $Ca(H_2PO_4)_2$, was applied. The experiments were carried out at the Connecticut Agricultural Experiment Station in the late spring and early summer of 1937.

Experiments

A fairly coarse, reddish-brown sand with angular grains, from a deep pit near New Haven, was used as culture medium. This was first washed in hot (65° C.) tap water, to remove most of the silt and soluble materials and to insure against damping-off (2). The wet sand was placed in glazed butter crocks each having a diameter of 17 cm., a depth of 10.5 cm., and a capacity of about 2500 ml. Every crock had a 5-mm. hole in the bottom to allow for constant-level subirrigation of the type described by DUNLAP and LIVINGSTON (3). The air-dry weight of the contents of each crock was approximately 3.7 kg. The crocks stood in tap water the surface of which was about 1 cm. above the bottom of the sand mass, which held about 750 ml. of water when in equilibrium with the free water below. For a series of cultures with seedlings of cabbage (*Brassica oleracea* L.), which will now be described, there were 9 crocks.

After the sand had been leveled off about 1.5 cm. below the crock rim, 3 of the 9 crocks (group *B*) each received 100 ml. of a 2-salt nutrient solution containing 0.01 gm. mol of KNO_3 and 0.001 gm. mol of $Ca(H_2PO_4)_2$. This solution had been found to produce as good growth of seedlings under these conditions as was obtained with more complex solutions. To the remaining 6 crocks no nutrient solution was added at the start. Three of them (group *A*) received no fertilizer at any time, but the remaining 3 (group *C*) subsequently received the addition just mentioned, 10 days after the seedlings had emerged.

Next, 200 seeds (germinative capacity about 80 per cent.) were planted in each of the 9 crocks and covered with about 0.5 cm. of washed sand, the surface being then sprinkled with water. The crocks stood about 8 cm.

apart on a watertight concrete bench with raised edge. This constituted the subirrigation tray, in which water was maintained at a depth of 1 to 2 cm. Thus the cultures were well subirrigated at all times. No artificial shade was used and on clear days, except for a few hours in early morning and late afternoon, the cultures were in direct sunlight.

In all cultures the seedlings emerged from the sand about 4 days after sowing. Ten days after their emergence most of those without fertilizer treatment (groups *A* and *C*) had only one very small true leaf, in addition to the cotyledons. The cotyledons were about 21 mm. above the sand, and the average fresh weight of aerial parts was about 8 gm. per 100 seedlings. At the same time, each of the seedlings of the initially fertilized cultures (group *B*) had a small true leaf; the cotyledons were about 50 mm. above the sand, and the average fresh weight of the aerial parts was about 25 gm. per 100.

Nutrient solution was at this time applied to 3 of the 6 previously unfertilized cultures (group *C*), as already mentioned, by sprinkling it over the seedlings and washing it into the sand by means of a light sprinkling of water. About 3 days later the seedlings that received this delayed application of fertilizer showed greatly accelerated growth and rapidly became darker green. They continued to grow vigorously until the cultures were discontinued on the 24th day after emergence. At that time it appeared that all cultures had reached a stage where any considerable further growth could not occur without further additional application of nutrient salts. Meanwhile, the seedlings that had received fertilizer treatment at the start, which

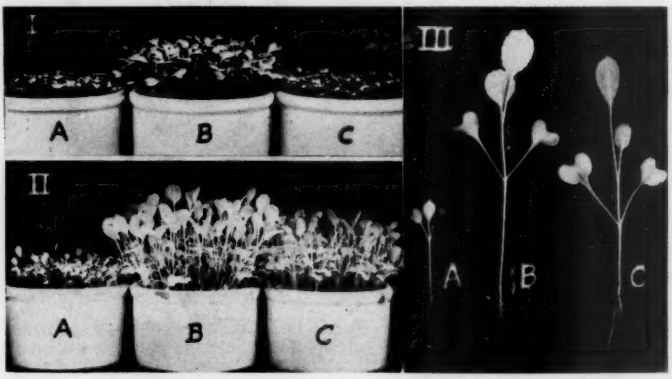

FIG. 1. Cabbage seedlings in sand cultures; I, 10 days after emergence; II, 20 days after emergence; III, representative seedlings 20 days after emergence. *A*, no nutrient salts applied at any time; *B*, KNO_3 and $Ca(H_2PO_4)_2$ added to sand when seeds were sown; *C*, the same salts added to sand 10 days after emergence of seedlings.

were already much advanced on the 10th day after emergence, continued to grow, but those without any fertilizer treatment grew only slightly, remaining very small—about as they were on the 10th day. The general appearance of the cultures on the 10th and 20th days after emergence and representative seedlings for the 20th day after emergence are shown in figure 1. Some growth data secured on the 10th and the 24th day after emergence are set forth by the diagrams of figure 2. For each time of observation these diagrams show the following features: Average height (millimeters) of cotyledons above sand; average length (millimeters) of stem above coty-

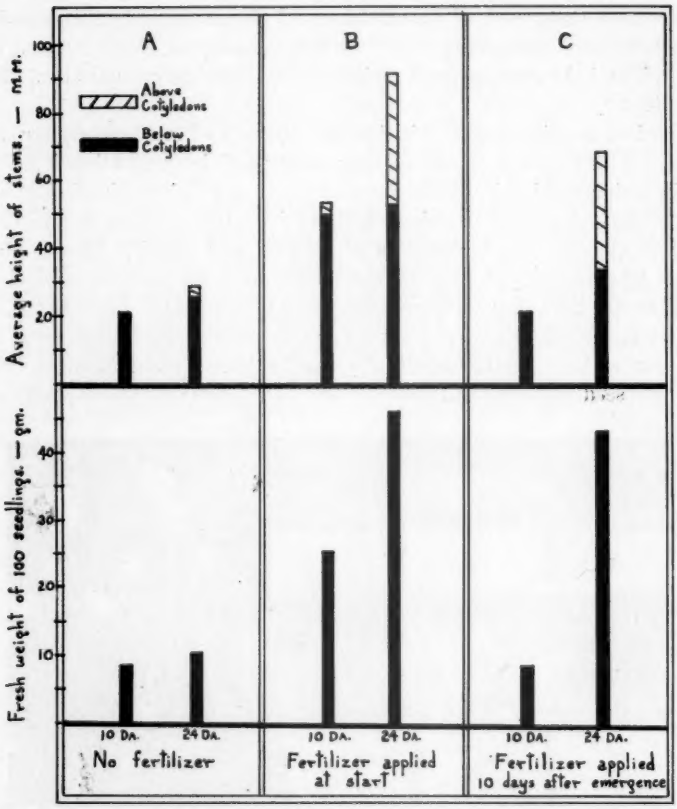

FIG. 2. Growth diagrams for seedlings represented in figure 1, showing length of stems and fresh weights on the 10th and 24th days after emergence of seedlings. Height of stem above cotyledons plus height of stem below cotyledons is total height of stem above sand.

ledons; average total height of stem—*i.e.*, average height (mm.) of terminal stem growing point above sand; and average fresh weight (gm.) of aerial portions per 100 seedlings.

At the end of the experimental period the seedlings showed marked differences according to the fertilizer treatments they had received. Those grown without any fertilizer (*A*) were very small indeed, with only a single leaf; those with initial fertilization (*B*) were apparently well grown but much "drawn" (as though somewhat etiolated); and those with delayed fertilization (*C*) were greatly superior—much stockier and shorter. Although both were grown in very dense stands, the seedlings of the *B* group were not well suited for transplanting into soil but those of the *C* group were in very excellent condition for transplanting.

Further examination of the figures furnishes a more detailed picture of these growth differences and the manner in which they were brought about.

In the first 10 days after emergence the height of the cotyledons above the sand became about 21 mm. in the absence of added nutrient salts (*A*) but it became about 50 mm. when the salts were present from the start (*B*). This height was subsequently increased from about 50 mm. only to about 53 mm. for the initially fertilized seedlings (*B*), and for those with delayed fertilization (*C*) this increase was greater; namely, from about 21 to about 33 mm. At the end of the experimental period the hypocotyls were very much shorter in the cultures with delayed fertilization (*C*) than in those with initial fertilization (*B*); indeed, with delayed fertilization the cotyledon height above the sand was at that time only about two-thirds as great as it had been with initial fertilization on the 10th day. It is thus clear that the final marked superiority and stockier appearance of the seedlings produced with delayed fertilization was in part due to this difference in hypocotyl elongation and final length.

The elongation of the stem above the cotyledons is also of interest. On the 10th day after emergence the average length of the entire epicotyl was only about 3 mm. with inital fertilization (*B*), and it was too short to measure when no fertilizer had been applied. In the following 14 days the length of the epicotyl increased from about 3 to about 39 mm. with initial fertilization, and from about zero to about 35 mm. with delayed fertilization. It thus came about that the total stem height above the sand was finally only about 68 mm. with delayed fertilization, while it was about 92 mm. with initial fertilization.

Although the seedlings with delayed fertilization were finally much shorter than those with initial fertilization, the final average fresh weight per 100 seedlings was almost as great with delayed fertilizer treatment (44 gm.) as with initial treatment (46 gm.). It is interesting to note that the fresh-weight value on the 10th day was only about 8 gm. for the *C* seedlings

while it was about 25 gm. for the *B* seedlings, and that these values increased during the next 14 days at different rates, attaining about the same magnitude on the 24th day. More than one-half of the final fresh weight of the initially fertilized seedlings represents growth during the first 10 days after emergence, but only about one-fifth of the final weight of the seedlings with delayed treatment represents growth before the nutrient salts were applied.

In summarizing, it may be said that delayed treatment was superior to initial treatment in that, although final fresh weight was about the same for both, final length of hypocotyl was much shorter and the length of the epicotyl was somewhat shorter with delayed treatment. Results similar to those just presented were sometimes obtained when the nutrient salts were applied 7 or 8 days after emergence of the seedlings from the sand, but when application was delayed only 3 or 4 days these effects were not produced. With longer periods, such as 20 days after emergence, more pronounced effects were obtained than with the 10-day period, and greater foliar development was noted on the seedlings with delayed fertilization.

Experiments similar to these were carried out with cucumber (*Cucumis sativus* L.) and tomato (*Lycopersicum esculentum* Mill.) and these plants were essentially like cabbage in their response to delayed application of nutrient salts.

Discussion

A physiological explanation of the results described above would surely involve consideration of growth-promoting substances and the relations of their actions to mineral nutrition and perhaps to light intensity and temperature of both air and soil. Under the conditions of the experiments with cabbage it appears that, in the absence of added salts (*A*), hypocotyl elongation practically ceased before the 10th day, but that when it ceased these organs still retained for a time their capacity to elongate further if salts were then supplied. An increment of about 12 mm. was added to the average hypocotyl length after the delayed application of nutrient salts on the 10th day, and that increment amounted to more than 36 per cent. of the final height of the cotyledons above the sand in the *C* cultures. When the added salts were present from the start (*B*), that increment was only about one-fourth as great (about 3 mm.), amounting to only about 6 per cent. of the final height of the cotyledons. It might be supposed that it was inadequacy of NO_3^- supply to hypocotyl and cotyledons which brought the period of hypocotyl elongation to a close somewhat before the 10th day in the *A* cultures, but it is difficult to imagine that hypocotyl elongation ceased for that reason in the *B* cultures, where the supply of that important ion was obviously still adequate for vigorous epicotyl growth throughout the succeeding 14 days.

Whatever conditions may have brought about the cessation of hypocotyl

elongation, it is clear that the average rate of that process in the first 10 days after emergence—and presumably throughout its whole grand period—was very much more rapid when the added salts were present than when they were not. This difference in rate may readily be supposed to have been caused by an accelerating influence exerted by the added salts, presumably by KNO_3 or by NO_3^-. That influence may have been effective directly on the elongating cells of the hypocotyl itself or indirectly through the cotyledons, or in both of these ways. VAN OVERBEEK (**5**) has recently reported that, when the cotyledons of radish seedlings (*Raphanus sativus* L., which is rather closely related to *Brassica*) were removed early, hypocotyl elongation was at first very slow, becoming more rapid and reaching its completion only after the epicotyls had attained considerable growth. He concluded that hypocotyl elongation could not occur in the usual way without the presence of a growth hormone, which appeared to be derived from the cotyledons or, in their absence, from the developing epicotyl. On the basis of that conclusion it may be supposed that the presumably salt-starved cabbage cotyledons of the present study were less effective to furnish the hypocotyls with requisite hormone than were the cotyledons of the initially fertilized cultures. Thus both the duration and the rate of hypocotyl elongation in the seedlings without initial fertilization may have been limited not only by inadequacy of NO_3^- supply but also by inadequacy of hormone supply. Furthermore, this supposed partial failure of the salt-starved cotyledons to deliver hormone to their hypocotyls may itself have been associated with NO_3^- deficiency in their own tissues, for AVERY, BURKHOLDER, and CREIGHTON (**1**) found that stem tips of sunflower and tobacco contained little or no growth hormone when inadequately supplied with NO_3^-, while the hormone content of these tissues was much greater when the supply of NO_3^- had been plentiful.

Elongation of the epicotyls began after hypocotyl elongation had practically ceased in the cultures without any fertilization, and it began somewhat earlier with the other treatments. Without added salts it began some time after the 10th day but the cultures with initial fertilization showed epicotyls about 3 mm. long on that day. In the succeeding 14 days the unfertilized epicotyls elongated very little (about 3 mm.) but those with initial and with delayed fertilization elongated rapidly, the rates being about equal. The final epicotyl length was somewhat greater with initial fertilization (about 39 mm.) than with delayed fertilization (about 35 mm.). The growing epicotyls probably produced their own growth hormones (**5**), but it does not appear that their rate of elongation was related in any clear way to the concomitant elongation of the hypocotyls, which had nearly completed their elongation before epicotyl elongation began. It is remarkable that epicotyl elongation in the final 14-day period was a little more rapid with initial fertilization than when the salts were added only at the beginning of that

period; although the initial supply of added nutrient salts had probably become more or less depleted by the 10th day, the residue was obviously sufficient for vigorous epicotyl development in the following 14 days, as has been noted. As far as the final stockiness of the seedlings with delayed fertilization was related to shortness of epicotyls, it was clearly due to earlier cessation of epicotyl elongation rather than to retardation of that process.

Of course the growth of these seedlings involved other features than those of hypocotyl and epicotyl elongation, notably, extensive development of the root system and foliar development and expansion. The superiority of the cultures with delayed fertilization was clearly ascribable, in considerable part, to their greater uniformity in size and more advanced leaf growth. The information at hand concerning these peculiarities is insufficient, however, to warrant any attempt to interpret them in terms of salt nutrition, hormones, etc. Nevertheless, the data presented in figure 2 concerning fresh weight of aerial parts (including hypocotyls, cotyledons, epicotyl stems, and leaves) may be examined briefly. Although the final average fresh-weight values derived from initial and from delayed fertilizer treatment are essentially alike (about 46 and 44 gm. per 100 seedlings), the rates of weight increase were very different. With initial fertilization the average fresh weight increased in the 14-day period from about 25 to about 46 gm., the increment being only about 21 gm., but when the salts were applied on the 10th day the corresponding increase was from about 8 to about 44 gm., the increment being about 36 gm. Of the final fresh weight, in the former case about 46 per cent., and in the latter about 82 per cent., had been produced in the last 14 days of growth. The seedlings produced by the method of delayed fertilization were therefore superior to those grown with initial fertilization in that a much greater proportion of their tissues had resulted from recent rapid development.

The results of the present study seem to lead to the suggestion that the stockier seedlings produced with the 10-day delay in fertilization might perhaps have possessed their superior characteristics to a still greater degree if they had been allowed to remain without fertilization somewhat more than the 10 days after emergence. Since it is reasonable to expect seedlings of different species or varieties to respond more or less differently to this technique of delayed fertilization, it seems likely that the delay requisite for most satisfactory results may be longer for some forms and shorter for others. A tentative rule may be proposed; to wit, that fertilizer salts are to be withheld for a while after hypocotyl elongation has ceased, until the hypocotyls have lost their physiological capacity to elongate further even after the salts are added. Also, other environmental features, besides fertilization and the time of its application, may be worthy of consideration as this technique is developed further; perhaps, for example, a suitably maintained low tem-

perature—of the sand or of both sand and air—throughout the preliminary period of salt starvation might give results even more satisfactory than those described in this paper. From the standpoint of practical horticulture as well as from that of plant physiology, this procedure of delayed fertilization with tray subirrigation seems highly promising and worthy of further experimental study.

Summary

1. This paper presents results of greenhouse experiments with cabbage seedlings closely grown in glazed crocks of washed sand with constant-level tray subirrigation. Some of the crocks were initially supplied with fertilizer salts (KNO_3 and $Ca(H_2PO_4)_2$); others received the same fertilizer treatment only on the 10th day after the seedlings had emerged from the substrate; and still other crocks received no fertilizer at any time. The experiments were discontinued on the 24th day after seedling emergence and the seedlings finally obtained differed markedly according to their fertilizer treatment.

2. Seedlings grown without any fertilization were obviously salt-starved, greatly stunted, with only a very small true leaf. Those that had received initial fertilization were well developed but they appeared as though partially etiolated, having long, slender hypocotyls and epicotyls; they were unsuited for transplanting. Those that had received delayed fertilization were like those with initial fertilization in fresh weight of aerial parts but each had relatively shorter hypocotyls and epicotyls. These seedlings with delayed fertilization were remarkably stocky and well suited for transplanting.

3. Physiological reasons for the pronounced superiority of the cultures with delayed fertilization are briefly considered, especially with reference to hypocotyl elongation, epicotyl development, and fresh weight of the aerial parts.

4. The procedure of delayed fertilization with washed sand and with tray irrigation is highly promising from the horticultural point of view, for it seems to offer, at least under greenhouse conditions similar to those of New Haven in spring and summer, a very simple and almost automatic means for quantity production of seedlings suitable for transplanting. This general procedure should be useful also in scientific studies on the relations of seedling development to environmental conditions and to hormone influences.

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ISOLATION OF A HEXOSEMONOPHOSPHATE FROM PEA LEAVES

W. Z. Hassid

Introduction

Harden and Young (**7, 8**) showed that phosphates accelerate the fermentation of sugars by yeast-juice and discovered hexosediphosphoric acid in the products resulting from such fermentation. Since that time the relation of phosphates to carbohydrate metabolism has been the subject of intensive study. The investigations of the several hexosephosphates isolated from yeast and muscle contributed much toward the knowledge of the complex carbohydrate metabolism of the animal body and served particularly to elucidate the chemistry of muscle.

In recent years data have been accumulated which indicate that hexosephosphates are also formed in the higher plants and that they play perhaps as important a part in the carbohydrate metabolism of the plant as they do in the animal body.

The fact that phosphates stimulate the respiratory sugar metabolism in higher plants suggests that the formation of phosphoric esters may occur as part of this process (**4, 10**). It is also known that when preparations of germinating peas, beans, barley, etc., are allowed to act upon the hexosediphosphoric acid of Harden and Young, products are formed which are characteristic of sugar breakdown in yeast or muscle (**1, 14, 15**). When phosphoglyceric acid is used as a substrate, pyruvic acid is formed (**16**).

Evidence that phosphorylation takes place in the plant was first presented by Bodnár (**5**). He demonstrated that inorganic phosphorus disappeared when added to ground peas in the presence of toluene. Barrenscheen and Albers (**2**) found that the acid-soluble phosphorus increased in irradiated *Elodea canadensis* and during germination of rye. Later Barrenscheen and Pany (**3**) showed the existence of hexosephosphates in *Elodea* after it was kept for a certain irradiation period in a dilute sugar solution to which inorganic phosphorus was added.

Tánkó (**23**), using pea preparations to which inorganic phosphorus was added, isolated a mixture of hexosediphosphate and hexosemonophosphate similar to the Harden and Young and the Robison esters. In addition, some esters were present which differed in constitution from the known phosphoric esters. Burkard and Neuberg (**6**) have shown that both glucose and fructose monophosphate are formed in beet leaves.

In the present work the writer also isolated a hexosephosphate from pea leaves, which is, apparently, a mixture of the glucose- and fructose-phosphoric esters. This affords more evidence to support the view that phos-

phorylation takes place in the higher plants with the production of phosphoric esters similar to those found in yeast or muscle, and also suggests a possible parallelism between carbohydrate metabolism in the higher plants and in yeast or muscle.

Experimentation

Isolation of the hexosemonophosphate

The plant material was prepared and the hexosephosphate isolated according to a slightly modified procedure described by Burkard and Neuberg (**6**).

Peas (variety Giant Stride) were planted on May 25, 1937, and harvested in July at about the time of the appearance of pods. Lots of 3 kg. of fresh leaves were quickly thrown into containers with 10 liters of boiling 95 per cent. alcohol, boiled for 15 minutes and allowed to stand overnight. The dark alcoholic solution was decanted, the leaves washed on a Büchner funnel with alcohol, dried at 28° C., and ground to a powder.

Two hundred and fifty gm. of the ground plant material were mixed with 5 liters of 0.4 N hydrochloric acid and stirred for 1½ hours. The solution was filtered off, cooled to 5° and neutralized with dilute sodium hydroxide to a weakly acidic reaction of pH 5 and then cooled again to the same temperature. One hundred and sixty cc. of 10 per cent. solution of copper sulphate and 500 cc. of calcium hydroxide were added and stirred for ten minutes. The precipitate was quickly removed by filtration, washed, and ground. It was then dissolved with dilute sulphuric acid, the acid being added until the solution gave a faintly blue color with congo red. During this operation the solution was kept cool by the addition of cracked ice to the mixture. The calcium sulphate formed was separated by filtration, the filtrate treated with hydrogen sulphide, the copper sulphide removed by filtration, and the hydrogen sulphide removed from the solution by a stream of air. To the liquid, which was kept ice-cooled, a warm saturated solution of barium hydroxide was added until it gave a red color to phenolphthalein; then carbon dioxide was introduced until the color disappeared. A 50 per cent. barium acetate solution was slowly added until no further precipitation occurred. The precipitate was filtered off and the liquid evaporated to about 100 cc. under reduced pressure at 40°, the solution filtered, and precipitated with basic lead acetate. The precipitate was washed on a Büchner funnel and then suspended in water. A stream of hydrogen sulphide was introduced into the solution, while the mixture was continuously shaken. The filtrate was cleared from the hydrogen sulphide by a stream of air, then barium hydroxide was added to the red end-point of phenolphthalein, after which it was filtered, and the solution precipitated with neutral lead acetate. This precipitate was filtered off and reserved for later treatment.

Basic lead acetate was added to the filtrate, and the precipitate, after being washed, was decomposed with hydrogen sulphide as before. The hydrogen-sulphide-free filtrate was neutralized again with barium hydroxide, and after filtration was added drop by drop to five times its volume of alcohol. The barium salt formed was filtered off on a sintered glass crucible, washed with alcohol, and dried in a desiccator. The precipitate previously obtained by the addition of neutral lead acetate was similarly treated.

About 0.8 gm. of the crude barium salt was obtained from two 250-gm. lots of the dried plant material.

This crude barium salt was dissolved in alcohol, 20 cc. of water was added, and the mixture shaken. The insoluble precipitate which separated out was filtered off, and the filtrate was treated with neutral lead acetate until no more cloudiness appeared. The precipitate was filtered off and the solution was treated with basic lead acetate. The lead salt formed was washed with water and changed into the barium salt in the manner described. It was precipitated with alcohol, dissolved in water, and precipitated again with alcohol. The yield of the barium hexosephosphate, after drying in vacuum at 70°, was 0.35 gm.

Analysis of the hexosephosphate

The barium salt of the hexosephosphoric acid ester was not obtained in the absolutely pure state.

[1]Found	C, 17.95 per cent.; P, 7.15 per cent.; Ba, 36.6 per cent.
Calculated for $C_6H_{10}O_8PO_4Ba$	C, 18.22 per cent.; P, 7.85 per cent.; Ba, 34.73 per cent.

The ratio of the Ba to P is therefore 1.155. Since only a limited amount of the material was available, further purification, according to the method of Robison (**21**), by conversion into the brucine salt and regeneration back into the barium salt was not attempted.

Burkard and Neuberg's ratio, Ba : P, for the barium hexosephosphate isolated from beet leaves was 1.336 (**6**). After regeneration from the brucine salt they obtained a Ba : P ratio of 1.063.

The specific rotation of the barium salt was determined by dissolving 10 mg. of the substance in a 2.5 cc. volumetric flask and polarizing in a 100-mm. (1 cc. capacity) tube. The reading was $-0.3°$. The specific rotation of the salt $[\alpha]_D$ was therefore of the order of magnitude of $-25°$.

The aldose value, determined iodimetrically according to the method of Macleod and Robison (**12**), was 11.8 per cent. and with the ferricyanide method (**9**) 38.0 per cent. The Seliwanoff reaction carried out on the substance as described by Roe (**22**) was positive. These values indicate that the carbohydrate fraction of the compound is a mixture of about 30 per cent. glucose and 70 per cent. fructose.

[1] The analyses were carried out semi-microchemically.

The negative rotation of the compound suggests that the fructose component might be the fructose-1-phosphate described by TÁNKÓ and ROBISON (**24**), as this is the only barium hexosephosphate with a negative rotation thus far reported.

An osazone was prepared from the barium salt as follows: 0.15 gm. of the salt was dissolved in 2 cc. of water, and the exact amount of dilute sulphuric acid necessary to precipitate the barium was added. The filtrate was treated in a test tube with 0.2 gm. of phenylhydrazine hydrochloride and 0.3 gm. of sodium acetate, and then heated on a water bath for half an hour. An osazone separated out on cooling. When recrystallized from a mixture of chloroform and alcohol, according to NEUBERG and REINFURTH (**20**), its melting point was 150°, which corresponds with values given in the literature.

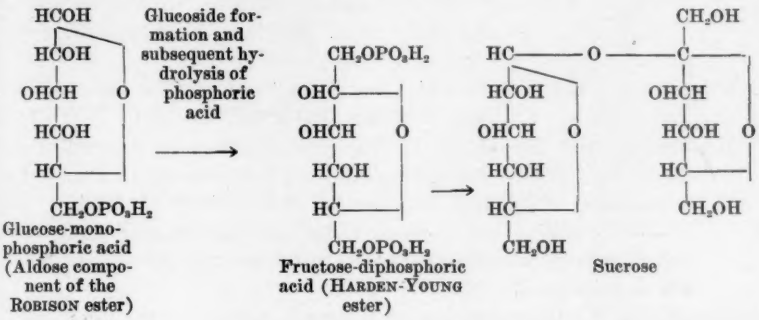
Discussion

NEUBERG and LEIBOWITZ (**17, 18, 19**) showed that when the proper enzyme acts upon either glucose or fructose ester an equilibrium mixture of glucose- and fructose-phosphoric acid ester results. The ease with which these two esters are formed, coupled with the fact that glucose- and fructose-phosphoric acid ester exist in actively metabolizing leaves of peas and sugar beets (**6**), which produce sucrose abundantly, suggests that phosphorylation of the hexose sugars is a necessary step for the synthesis of sucrose. Furthermore, the chemical structure of sucrose, containing the furanose (unstable or γ form) form of fructose and the stable pyranose form of glucose in its molecule, can be adequately explained only by assuming that phosphorylation of these two monosaccharides takes place before the synthesis of the sucrose molecule.

The fructosediphosphoric as well as the fructosemonophosphoric acid ester are known to possess the furanose structure. MORGAN (**13**) showed that the same methylhexosediphosphoric acids are obtained whether the methylation is carried out at room temperature or at 60°, the latter condition being favorable to the formation of methylfructopyranose, containing the stable 2:6 or amylene oxide ring. MORGAN and ROBISON concluded from these facts that the second phosphoric acid group is attached to the terminal C atom, thereby preventing the formation of the stable 2:6 oxide ring. LEVENE and RAYMOND (**11**) arrived at a similar conclusion from the results of methylation experiments. Phosphorylation of the fructose molecule evidently takes place when it is in the straight chain form. Subsequently, when the ring is formed, it results in a furanose structure because the hydroxyl of the sixth carbon atom is occupied in the phosphoric acid ester linkage, thus preventing the formation of the pyranose structure.

Now, if we assume a direct glucosidal linkage between this five-mem-

bered ring of phosphorylated fructose and the six-membered glucose-monophosphoric acid and then subsequent hydrolysis of the phosphoric acid groups, the molecule of sucrose would result:


Glucoside formation and subsequent hydrolysis of phosphoric acid
Glucose-monophosphoric acid (Aldose component of the ROBISON ester)
Fructose-diphosphoric acid (HARDEN-YOUNG ester)
Sucrose

Summary

1. A hexose monophosphate was isolated from pea leaves, which proved to be a mixture of glucose- and fructose-phosphoric acid esters. The negative rotation of the barium salt of this hexosephosphoric acid seems to indicate that the fructose component is, probably, fructose-1-phosphate.

2. It is suggested that phosphorylation of the glucose and fructose components is a necessary step in the synthesis of sucrose in the plant.

3. The occurrence of the unstable furanose form of fructose in the sucrose molecule is explained by assuming that phosphorylation of the fructose and glucose takes place prior to the synthesis of sucrose in the plant.

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INFLUENCE OF OSMOTIC PRESSURE ON SPORULATION BY *BACILLUS SUBTILIS*

JAMES L. ROBERTS, WELDON C. WHITE AND ELIZABETH OJERHOLM

(WITH TWO FIGURES)

Studies on the physiology of bacterial endospore formation have in many cases involved the addition of one or more compounds to a basal medium, and a determination of the effect of these upon the percentage of cells appearing as spores. The consequences, so far as the spore crop is concerned, of the addition of electrolytes (FITZ-GERALD, **5**; FABIAN and BRYAN, **3**), fermentable carbohydrates (ESTY, **2**; DE SMIDT, **7**), vapors of fat solvents (MICHAILOWSKY, **6**), antiseptics, and many other compounds have been studied by this method.

Among the bacteria, forms can be found which are more resistant to great changes in osmotic pressure than are any other forms of life (FALK, **4**). Many species of bacteria, however, in common with other plants and animals, are readily affected by changes in the osmotic pressure of their menstruum (CURRAN, **1**). It is well known that certain physical properties of a medium may influence the degree of endospore formation occurring within that medium. According to our best knowledge, little or no work has been done to determine the influence of osmotic pressure on sporulation. Studies involving the addition of materials of small molecular size could be more easily interpreted if there were available more definite knowledge of the effect of osmotic pressure on spore formation. The present work was undertaken to determine whether osmotic pressure is a factor sufficiently important to warrant consideration in future studies of bacterial sporulation.

Methods

A 0.5 per cent. Bacto-peptone water was used as a basal medium to which the various materials used in raising the osmotic pressure were added. The basal solution was prepared in one large vessel and distributed in 25-cc. volumes into 6-oz. prescription bottles with screw caps. The following materials were then weighed or measured into the bottles of basal solution:

1. Ten concentrations of KCl (0.05 M to 0.5 M)
2. Ten concentrations of NaCl (0.05 M to 0.5 M)
3. Ten concentrations of $MgSO_4$
4. Ten concentrations of $MgCl_2$ (0.033 M to 0.333 M)
5. Ten concentrations of $CaCl_2$ (0.033 M to 0.333 M)
6. Ten concentrations of sodium acetate (0.05 M to 0.5 M)
7. Ten concentrations of glycerol (0.1 M to 1 M)

8. Six concentrations of lactose (0.1 M to 0.6 M)
9. Ten concentrations of agar (0.01 to 0.1 per cent.)
10. Ten concentrations or NaCl (0.05 M to 0.5 M) in basal medium with 0.1 per cent. agar

The media were sterilized in 15 pounds of steam pressure for 30 minutes and the hydrogen-ion concentrations of the various solutions were then ascertained to be between pH 6.4 and 6.6. Inoculation was accomplished by two drops of a 24-hour peptone water culture of the "K strain" of *Bacillus subtilis* obtained from the culture collection of the University of Texas. The cultures were incubated in a horizontal position with the bottle caps loosened. The incubation temperature used was 37° C. Under these conditions there was little or no pellicle formation.

Smears were prepared from each of the cultures after two and four days of incubation. It was intended that the final pH and osmotic pressure values should be determined at the completion of four days of incubation, but time did not permit a determination of these until the eighth day.

The smears were steamed in 5 per cent. aqueous malachite green, destained with distilled water, and counterstained with 5 per cent. mercurochrome. Approximately 500 cells were counted on each smear, and from these the percentage of spores was calculated.

In general, growth was not visibly inhibited by the osmotic pressures of the solutions we have used in these studies. Osmotic pressures greater than 23 atm. have not been investigated since only rarely do media contain sufficient osmotically active materials to produce greater pressures.

Results

As would be expected, the pH of most of the media became increasingly alkaline to 7.5 to 8.0 during eight days of growth. Those media containing glycerol and lactose were exceptions in that they became slightly more acid than the uninoculated control media. Because the results of spore counts in media containing glycerol and lactose may not be comparable with the results obtained in the remainder of the test solutions, they are omitted in future considerations. The percentage of sporulation in the presence of each of these two materials was lower than with the other chemicals tested.

Growth of *B. subtilis* seemed to cause a slight increase in the osmotic pressure of the menstruum after eight days of incubation. Since this change was always less than one atmosphere, it is considered negligible.

The curves representing sporulation in any series of media after two and four days of incubation (fig. 1) are sufficiently similar to indicate no excessively great experimental error in the mechanics of the determinations. The gravest danger is in drawing conclusions when so few compounds have been studied, and when it has been impossible to completely isolate the effects

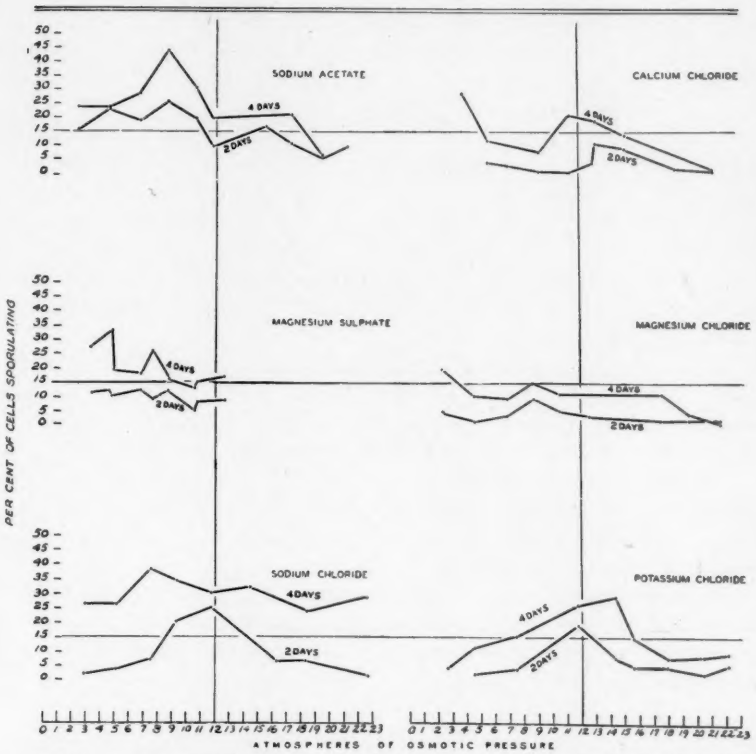

FIG. 1. Sporulation by *Bacillus subtilis* in peptone broth as influenced by variously induced osmotic pressures.

of the osmotic pressure from the specific effects of the added chemicals. Specific influences of the chemicals are evident in the results presented in figure 1. It will be noted, however, that the various curves have some characteristics in common. In view of this, it seemed both fair and helpful to compound the results from all the media plotted in figure 1. The resulting histogram is shown in figure 2.

The percentages of sporulating cells in media containing agar are much higher than in other media. Therefore, the results with agar are of little value for comparison and are omitted from figures 1 and 2. The osmotic pressure of media containing agar has little or no effect upon sporulation.

The influence of osmotic pressure, at least in the case of *B. subtilis*, seems not to be sufficiently great to warrant serious attention in future spore studies. As shown in figure 2, the percentages of sporulation in media with osmotic pressures ranging from 2 to 18 atm. are not significantly different.

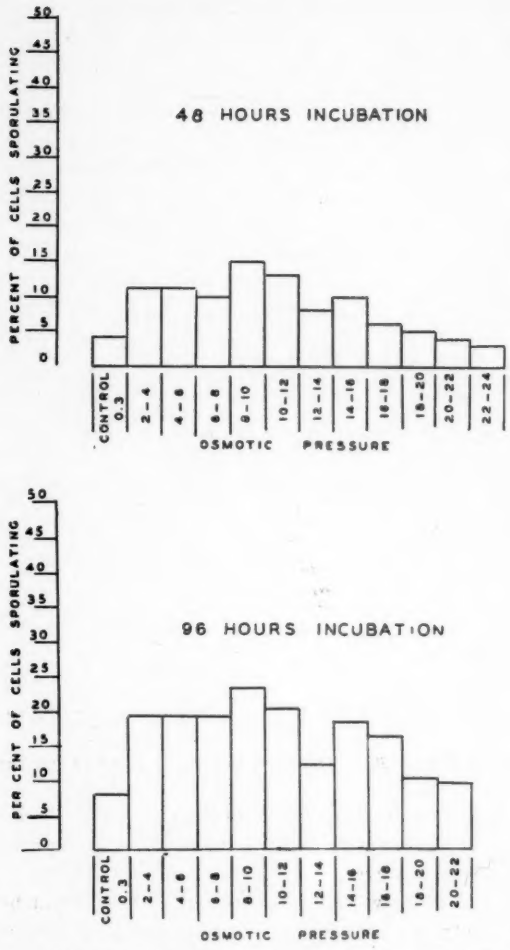

FIG. 2. Composite results (excepting those from media containing agar) showing effect of osmotic pressure on sporulation by *B. subtilis*.

There does seem to be a slight inhibition of sporulation by pressures above 18 atmospheres and below 2 atmospheres.

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BRIEF PAPERS

INFLUENCE OF THE LENGTH OF DAY ON THE DORMANCY OF TREE SEEDLINGS

FELIX G. GUSTAFSON

(WITH ONE FIGURE)

Since the introduction of the term "photoperiodism" by GARNER and ALLARD (**2**), 18 years ago, almost all of the work on the subject has dealt with reproductive responses of plants to length of day, and only very little with vegetative responses. In fact, until recently it was generally considered that photoperiodism had reference only to sexual responses. In 1923 (**3**) GARNER and ALLARD, among their other experiments, made some preliminary observations on the retention of leaves by *Rhus glabra* and *R. copallina* when kept under long days. They found that these plants retained their leaves longer under long days than under short days, but eventually shed them even under long day conditions. They also noted that *Liriodendron tulipifera* plants grown in the greenhouse produced new leaves earlier under long day conditions than under short day conditions. BOGDANOV (**1**) working near Leningrad shortened the photoperiod of 1- and 2-year-old seedlings of several tree species and found that this treatment shortened their seasonal growth period. MOSHKOV (**7**), also working near Leningrad, found the same to be true for the plants he studied. He also found that, though a shorter than normal photoperiod shortened the seasonal period of growth, the total amount of growth made by the plant per growing season was not always decreased. Thus *Pyrus ussuriensis* and *Salix babylonica* grew more under 10, 12, and 14 hours of daylight than under the normal 20-hour day. There were also morphological changes brought about by changing the photoperiod. The early cessation of growth under the shorter than normal photoperiod enabled the wood to ripen and the plants were more frost resistant than plants grown under normal length of day.

From his work with several species of trees, KRAMER (**5**) came to the conclusion: "variation in length of day may, to a considerable extent, control the duration of the growing season of certain tree species." In a later paper KRAMER (**6**) describes a situation in which a street light caused an increased growth period in shrubs; this continued growth prevented the wood from maturing, and the stems were winter killed.

Several years ago the writer became interested in the vegetative response of tree seedlings to length of day. At that time some 3-year-old transplants of *Picea canadensis* and *Pinus resinosa* were brought into the laboratory early in the fall, and potted for class use. After the class was through with

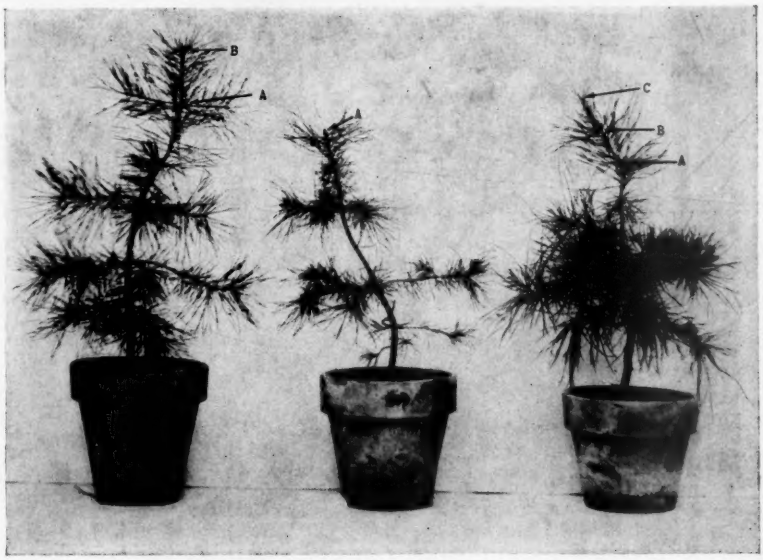
the plants, they were kept in the greenhouse for possible use in the spring semester. A relatively short time after the plants were brought into the greenhouse the white spruce plants began to grow and made an elongation of several centimeters, but the pines showed no sign of growth activity. Sometime in February or March a few of the dormant pine seedlings were placed under 500-watt lamps and given a photoperiod of about 16 hours. Within a few weeks these plants commenced to grow, but the plants receiving no additional light remained dormant. In fact, the plants given the normal day length did not grow in length during all of the following summer. As only a few plants were used, no conclusions could be drawn from the experiments and no further observations were made for 5 years.

Early in the fall of 1935 one hundred 3-year-old *Pinus resinosa* plants similar to the ones mentioned above were brought into the greenhouse and potted in 4-inch pots. These plants remained in a low-temperature greenhouse during the winter of 1935–1936. The following summer the plants were repotted into 6-inch pots and placed back in the same greenhouse, but they made no growth in length during this period, and many died. Early in the fall of 1936 twenty-five of these plants were put into a cold frame. They were left there until March 5, 1937, when they, together with the remaining plants that had not been exposed to the freezing temperature were placed in a warm greenhouse. Of the hundred plants that were potted in 1935 there were only 39 good plants left; of these 14 had been exposed to a freezing temperature, and 25 had remained at a temperature of from 10° to 12° C. From the latter group 14 plants were selected, and, together with the 14 plants that had been allowed to freeze, were placed in a part of the greenhouse where they received only the natural length of day. The remaining 11 plants of the 25 were placed under 500-watt lamps and were given a photoperiod of about 16 hours. The temperature was the same for the two lots of plants.

On May 21, the plants were examined. The buds were counted on each plant and those that had opened were also counted. On the non-frozen plants under the short day conditions, 29.5 per cent. of all the buds showed a development of one or more needles. In most of the buds only a few needles were produced and there was no elongation of the stem. Only 6 buds out of a total of 261 grew as much as 1 cm. On the corresponding plants given the long days, 87.5 per cent. of all of the buds showed some growth. Every apical bud, and on most plants at least two other buds, grew as much as one centimeter in length. The plants that had been exposed to the outdoor temperature until March 5 and normal greenhouse conditions with short days since that time had 87.6 per cent. of all buds open. This is the same as for the plants under the light. Every apical bud developed and in most plants 2 other buds showed considerable growth. The total

growth in length was somewhat greater than that made by the plants under the lamps.

Both lots of plants receiving a normal photoperiod became dormant after whatever growth they had made during the first 2 months in the warm greenhouse, and they remained dormant until the middle of the summer when they were discarded. On the other hand, the plants exposed to the 16-hour photoperiod produced a number of buds and many of these made a second growth during the early summer (fig. 1, plant 3).



1 2 3

FIG. 1. *Pinus resinosa.* The three plants are of the same age and were treated alike until the fall of 1936 when plant 1 was placed in a cold frame and 2 and 3 left in a cool greenhouse. On March 5, plants 1 and 2 were brought into a warm greenhouse, and plant 3 was placed in another warm greenhouse and supplied with additional light, giving it a photoperiod of approximately 16 hours. Plant 1 grew the amount indicated between A and B; plant 2 did not grow at all; plant 3 went through two growth cycles (first it grew from A to B, after which, in the early summer, the distance B to C).

Summary

It has been found that if *Pinus resinosa* seedlings are not exposed to freezing conditions during the winter, they make either no growth or only a very slight growth during the summer, unless exposed to a photoperiod of about 16 hours. If this is found to be true in nature, we would expect to find *Pinus resinosa* limited to regions where freezing occurs during the

winter. Some of the pine seedlings had two growth cycles when exposed to the long photoperiod.

The writer is interested in hearing from anyone who has first-hand information concerning the presence of *Pinus resinosa* in regions where there is no freezing temperature.

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EFFECT OF DROUGHT ON PROTOPLASMIC ELASTICITY[1]

HENRY T. NORTHEN

It is generally believed that the capacity of a plant to resist drought is determined to some extent by the nature of the protoplasm. The data in this paper indicate that the elasticity of protoplasm in cells of rye coleoptiles increases when moisture is deficient.

Seeds of spring rye were sowed in two flats. After sowing, both flats were thoroughly watered. The soil in one flat was then allowed to dry out, whereas the soil in the other flat was kept constantly moist. On the seventh and succeeding days after sowing, 6 plants were removed from each flat.

[1] Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 165.

After the root systems and leaves which were emerging from the coleoptiles were cut off, the coleoptiles with enclosed leaves were centrifuged. Following centrifugation the responses of the chloroplasts in cells of the coleoptiles were noted. On the eighth and ninth days samples of soil were removed from each flat, weighed, allowed to dry at room temperature, and the moisture percentages on a dry weight basis were calculated. The data are summarized in table I, in which + indicates that the chloroplasts had been moved by the centrifugal acceleration whereas 0 indicates that the chloroplasts had not been moved.

TABLE I

EFFECT OF DROUGHT ON PROTOPLASMIC ELASTICITY

DAYS AFTER SOWING	SOIL MOISTURE	LEAVES	RESPONSE OF CHLOROPLASTS TO CENTRIFUGATIONS OF			
			680 × GRAVITY FOR ONE MINUTE		170 × GRAVITY FOR FOUR MINUTES	
			IN CELLS NEXT TO VEIN	IN MORE DISTANT CELLS	IN CELLS NEXT TO VEIN	IN MORE DISTANT CELLS
7	Slightly dry	Turgid	+	+	+	+
7	Moist (control)	"	+	+	+	+
8	5.9%	Slightly wilted	+	+	0	0
8	18.8 (control)	Turgid	+	+	+	0
9	2.8%	Slightly wilted	0	0	0	0
9	17.1 (control)	Turgid	+	+	+	+
10	Moist*	Turgid	+	0	+	0
10	Moist (control)	"	+	+	+	0
11	Moist*	Turgid	+	+	+	0
11	Moist (control)	"	+	+	+	0

* After the experiment on the ninth day was completed the dry flat of plants was thoroughly watered.

NORTHEN (**1, 2**) has previously demonstrated that protoplasm in cells of *Zygnema* and *Spirogyra* is an anomalous fluid and hence is probably built up of intermeshed linear molecules or micelles. In *Spirogyra* the velocity with which the chloroplasts moved in response to centrifugal accelerations was approximately governed by the following equation:

$$V = k(c - c_o)$$

in which V is the velocity of chloroplastic movement, k a constant, c the centrifugal acceleration used, and c_o is the initial starting centrifugal acceleration at which, or below which, the chloroplasts will not move regardless of how long the acceleration is allowed to act.

The data in table I support NORTHEN's conclusion that protoplasm is an

anomalous elastic fluid. If protoplasm in cells of rye coleoptiles be a true fluid the chloroplasts should have been displaced as much with a centrifugation of 170 × gravity for 4 minutes as with a centrifugation of 680 × gravity for 1 minute. In several instances (table I) the chloroplasts were displaced in cells distant from the veins by an acceleration of 680 × gravity but were not displaced by an acceleration of 170 × gravity. In such instances the initial starting acceleration (c_o) for such cells was equal to or greater than 170 × gravity. Hence when an acceleration of 170 × gravity was used:

$$V = k(170 - 170) = 0 \quad \text{or} \quad V = k(170 - > 170) = < 0.$$

In some instances, however, the chloroplasts were displaced by both accelerations. At such times the value of c_o was less than 170 × gravity. NORTHEN (2) has found that in *Spirogyra* the value of c_o varies from day to day.

The data in table I indicate that a deficiency of water caused an increase in the value of the initial starting acceleration (c_o), which was interpreted as an increase in protoplasmic elasticity. For example, on the ninth day the value of c_o in cells with a deficiency of water was equal to or greater than 680 × gravity whereas for cells with sufficient water the value of c_o was less than 170 × gravity. Hence, for cells with a deficiency of water the velocity of chloroplastic movement when an acceleration of 680 × gravity was used would be:

$$V = k(680 - 680) = 0 \quad \text{or} \quad V = k(680 - > 680) = < 0.$$

However, for plants with sufficient water the velocity would be:

$$V = k(680 - < 170) = k(> 510).$$

The higher the value of c_o the greater the elasticity of the protoplasm and hence the more gel-like would be the protoplasm. Furthermore, it is highly probable that the more gel-like the protoplasm the more avidly it will retain water.

It will be noted (table I) that the changes in elasticity occurred in a relatively short time. For example, the elasticity of the protoplasm had returned to normal in cells adjacent to the veins 24 hours after the plants were watered and in all cells 48 hours after watering. The rapidity with which the changes in elasticity occurred suggest that in this experiment the changes in elasticity were due to orientations of existing molecules or micelles rather than to the formation of new substances or the increase in the amount of such substances as hemicelluloses.

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NOTES

Annual Election.—The fifteenth annual election of the American Society of Plant Physiologists, held during May and June, 1938, resulted in the choice of the following officers for 1938–1939: President, Dr. WALTER F. LOEHWING, State University of Iowa; vice president, Dr. GEORGE W. SCARTH, McGill University; elected member of the executive committee (term to expire in 1941), Dr. E. J. KRAUS, University of Chicago; elected member of the editorial committee (term to expire in 1941), Dr. EDWIN C. MILLER, Kansas State Agricultural College. Many opportunities for service will be presented to the membership of the Society, and the officers will appreciate the cooperation of all members in promoting the welfare and objectives of the organization. The immediate objective is preparation for the Richmond meeting in December, 1938. Prompt response to requests for service in connection with programs and committee assignments will lighten the tasks of those responsible for the program as a whole.

New England Section Meeting.—Although the New England Section held its meeting far from the center of distribution of its membership, and some members had to travel several hundred miles to attend, yet the meeting on the Maine Campus at Orono, Maine, was one of the best meetings the section has had. The attendance equaled or possibly exceeded that of previous meetings, and afforded evidence of the cooperation and friendship which annual sectional meetings can establish. It was a pleasure to all attendants to participate in the hospitality of Vacationland.

In addition to the program of papers, there was a symposium on the physiology of aquatic plants, with Dr. C. W. MUENSCHER, of Cornell, and Dr. C. COTTAM, of the United States Biological Survey, as guest speakers. Also, a special meeting dealing with the physiological problems of blueberry culture preceded the general sectional activities.

A report by Dr. J. C. LYON, Dartmouth, on the symposium at Indianapolis on methods of teaching plant physiology, stimulated sectional interest in a continuation of this work by a committee of which Dr. LYON is chairman.

The section interested itself in the problems of chemurgy, and organized a committee with Dr. C. G. DEUBER as chairman to consider the objectives of chemurgy and the relation of plant physiology to the attainment of those objectives.

The annual dinner was held on the evening of May 13, in Merrill Hall. In addition to the address of welcome by President ARTHUR A. HAUCK, there was a color motion picture on the Wild Life of Maine by JOSEPH S. STICKNEY, Warden Supervisor of the State Department of Inland Fisheries and Game.

Officers for the following year were elected, Dr. HUBERT BRADFORD VICKERY, of the Connecticut Agricultural Experiment Station, being chosen as chairman of the section. Dr. CHARLES J. LYON is vice-chairman, and Dr. LINUS H. JONES was re-elected secretary-treasurer.

The meeting in May, 1939, is scheduled to be held in New Haven. With several years of most successful operation, the New England Section is firmly established as part of the scientific progress of the region.

Western Section.—The meeting of the Western Section of the American Society of Plant Physiologists, held with the Pacific Division of the A.A.A.S. in Balboa Park, San Diego, was particularly successful this year. A four-day program offered three sessions for submitted papers, four joint symposia, and several festive occasions. The sessions were held in the Floral Association Building, in cool, well-ventilated, amply equipped rooms.

The session of Tuesday morning, June 21, was devoted to submitted papers on auxins, bud inhibition, polar transport, autonomic exudation cycle, and responses to ethylene. In the afternoon, a joint symposium with the Western Society of Soil Science considered salinity problems: Salt absorption and transport, by D. R. HOAGLAND and T. C. BROYER; toxicity and accumulation of chloride and sulphate salts, by F. M. EATON; and the program of the U. S. Regional Salinity Laboratory, by O. C. MAGISTAD. This symposium on salt tolerance emphasized the growing importance of salinity problems in irrigation agriculture, and the part plant physiology must play in the solution of these problems.

On Wednesday morning plant invasion on the Pacific coast was the topic of a joint session with the Botanical Society. Starting with a discussion of early introductions as revealed by study of plant remains in ancient adobe bricks, G. W. HENDRY and J. N. BOWMAN gave a vivid portrayal of the influence of the coming of the white man on plant succession in the west. H. L. MASON described the source and distribution of recent plant invaders; and C. J. KRAEBEL discussed the use of introduced species in planting road grades and burned areas in forests. M. W. TALBOT directed attention to the extent to which introduced species have invaded valley and foothill pastures in California. In some instances the invaders may form 70 per cent. of the present vegetation. Some of the invaders are beneficial, others weedy and detrimental. W. W. ROBBINS discussed the weed losses of agricultural areas.

A symposium on cell wall structure, Wednesday afternoon, included papers by W. H. DORE, O. L. SPONSLER, JAMES BONNER, W. S. STEWART, and A. S. CRAFTS. The papers presented the development of cell walls from the simplest molecular building units to their final rôle in the functioning plant. Particularly interesting and valuable were the results of the use of modern chemical, x-ray, and polarization methods of studying cell wall structure and the relation of structure to the attendant growth processes.

The Thursday morning session included papers on weed control, toxicity of insecticides in the soil, and plant-soil relations. The afternoon symposium on progress in plant science had noteworthy summaries on plant hormones by F. W. WENT; permeability, by L. R. BLINKS; dynamics of photosynthesis by W. ARNOLD; and nature of viruses, by T. E. RAWLINS.

The final session on Friday dealt with plant biochemistry and mineral nutrition, and revealed the progress being made with reference to the rôle of metals in the nutrition of plants.

The dinner for plant physiologists was held Thursday evening. Officers for the following year are: Chairman, F. W. EATON; vice-chairman, J. R. FURR; secretary, A. S. CRAFTS.

Life Membership Committee.—The fifteenth award of the CHARLES REID BARNES life membership will be made at the Richmond meeting. According to the constitution and recent ruling by the executive committee, this award is to be made to some prominent foreign plant physiologist. The committee as announced by president LOEHWING is constituted as follows: Chairman, Dr. R. H. TRUE, University of Pennsylvania; E. C. AUCHTER, U. S. Department of Agriculture; D. R. HOAGLAND, University of California; F. M. ANDREWS, Indiana University; and SAM F. TRELEASE, Columbia University.

Stephen Hales Award.—The sixth award of the STEPHEN HALES prize is also to be made during the annual meeting at Richmond. Dr. HUBERT BRADFORD VICKERY, of the Connecticut Agricultural Experiment Station, is chairman of the award committee, with Dr. C. A. SHULL, the University of Chicago, and Dr. K. V. THIMANN, Harvard, as members of the committee. By tradition the announcement of these awards is made at the annual dinner, which is thus made one of the happiest features of the social activities of the Society. It is hoped that all plant physiologists attending the meetings will find it possible to attend the annual dinner and share in the pleasures of that occasion.

Florence Brown Charlton.—With deep regret we record the death on March 31, 1938, of Mrs. Florence Brown Charlton, a member of the Society, wife of Mr. CLARENCE D. CHARLTON, at La Salle, Illinois. Mrs. CHARLTON was born in Chicago on December 4, 1904, and was educated in the Chicago public schools. After graduation at the Englewood High School, Chicago, in 1921, she matriculated at the University of Chicago, where she specialized in botany and plant physiology for 5 years. She was repeatedly awarded honors and scholarships for excellence of her work in the junior and senior colleges, and was graduated with the S.B. degree with Phi Beta Kappa honors in June, 1925.

Continuing her work in the graduate school, and majoring in plant physiology, she won her M.S. degree in 1926, and was elected to associate membership in Sigma Xi. On September 1, 1926, she became a graduate assistant at the Pennsylvania State College, which institution she served faithfully for 10 years. She was advanced to the rank of assistant in botany on October 1, 1927, and to an instructorship on July 1, 1928. Because of local rulings that persons above the rank of instructor could not obtain advanced degrees at the Pennsylvania State College while a member of the college staff, she remained in the instructorship rank, and worked for her Ph.D. degree, which was granted her posthumously at the June, 1938, convocation. She taught general botany, and assisted Dr. W. H. POPP in plant physiology, was always an excellent teacher, a thorough and hard-working student, a very capable investigator. She was joint author with Dr. POPP of several valuable contributions and reviews on ultraviolet and visible radiations as factors in plant response. Her thesis was published in the July, 1938, number of the American Journal of Botany.

She was a member of the Botanical Society of America, and the Pennsylvania Academy of Science, as well as of the American Society of Plant Physiologists.

In June, 1936, she resigned her position at the Pennsylvania State College to engage in a career of home-making, a career in which she found great happiness. To retain her interest in plant physiology, she had been engaged temporarily as assistant editor of PLANT PHYSIOLOGY, and gave very valuable assistance with the October number of 1937. In recognition of her unique qualifications for this work, her command of English, and her sense of organization, arrangements had been concluded to have her begin a permanent connection with the editorial office on July 1, 1938. Action had been approved by the executive committee at the Indianapolis meeting, looking toward this end.

The immediate cause of her death was failure to rally after a Caesarean operation on March 27 for the birth of a daughter. Blood transfusions were given, but failed to save her. She had a fine clear mind, was exceptionally competent in all of her services, possessed a rare and beautiful personality, and a character above any possible reproach. Such personalities can never be replaced in kind, and her loss is felt most keenly by all who had the privilege of knowing her and working with her. Our sympathy is extended particularly to her bereaved husband and his family.

Annual Review of Biochemistry.—The preparation and publication of the Annual Revew of Biochemistry is an outstanding achievement which places all biochemists and physiologists under a debt of gratitude to the

editors, reviewers, and publishers of this annual volume. Volume VII is somewhat smaller than its immediate predecessor because some of the reviews contemplated did not come through for publication. The majority of the reviews cover the same fields as were covered last year. This is as it should be, for we need the summaries of active fields repeated year after year. A few new subjects of timely interest are treated in this volume.

The reviews which are repeated, with or without subdivisions of the previous treatment, and with different authorship, are as follows: Biological oxidations and reductions, by L. MICHAELIS and C. V. SMYTHE; chemistry of the crystalline enzymes, by J. H. NORTHROP and R. M. HERRIOTT; the chemistry of the carbohydrates and glycosides, by E. F. ARMSTRONG; the chemistry of the acyclic constituents of natural fats and oils, by G. S. JAMIESON; the chemistry of amino acids and proteins, by M. BERGMANN and C. NIEMANN; the chemistry and metabolism of the compounds of phosphorus, by K. LOHMANN; carbohydrate metabolism, by H. E. HIMWICH; fat metabolism, by F. VERZAR; metabolism of amino acids and proteins, by H. A. KREBS; the hormones, by O. WINTERSTEINER and P. E. SMITH; the vitamin B group, by R. A. PETERS and J. R. O'BRIEN; vitamin C (ascorbic acid, cevitamic acid), by R. A. PETERS and H. W. DAVENPORT; the fat-soluble vitamins, by J. C. DRUMMOND; nutrition, by H. H. MITCHELL; the biochemistry of muscle, by E. LUNDSGAARD; the chemistry of bacteria, by W. H. PETERSON and M. J. JOHNSON. In addition, there are seven titles, as follows: The metabolism of creatine and creatinine, by K. THOMAS; acid-base metabolism, by J. SENDROY, Jr.; liver and bile, by A. C. IVY and L. A. CRANDALL, Jr.; animal pigments, by R. LEMBERG; the terpenes, saponins, and closely related compounds, by W. A. JACOBS and R. C. ELDERFIELD; organic insecticides, by F. B. LAFORGE and L. N. MARKWOOD; and growth regulators in the higher plants, by P. BOYSEN JENSEN.

The reviews are excellent surveys of the fields covered, and are complete enough to keep the reader who depends upon them well informed as to the current trends of research, and the philosophy of biochemical interpretation. Year by year the *Annual Review of Biochemistry* has made itself a dependable source of authentic information, and it is recognized as an invaluable and indispensable part of the equipment of the biochemist and physiological investigator. With author and subject indices, the new volume contains 571 pages and retails at $5.00 per copy. It can be ordered from Annual Reviews, Inc., Stanford University, California. The press work by the Stanford University Press is excellent.

It seems appropriate to call attention now to the fact that an *Annual Review of Physiology* is to be added to the publication activities of Annual Reviews, Inc., with cooperation of the American Physiological Society. This will permit separation of the reviews that are primarily physiological from

those that are primarily biochemical in nature. It is hoped that the new departure will be as successful, and as useful to investigators and teachers as the *Annual Review of Biochemistry* has become.

General Plant Physiology.—A new text book of plant physiology comes from the press of Williams and Norgate Ltd., London. The author is E. C. Barton Wright, well known to his American friends as the author of *Recent Advances in Plant Physiology*, published by Blakistons in 1930 and 1933. The new *General Plant Physiology* is divided into three parts: The general physiology of the cell; metabolism; and growth, reproduction and irritability. The six chapter headings of the first part are: The scope of physiology; colloids and protoplasm; osmotic pressure and the water relations of the plant; permeability; transpiration; and the ascent of sap. The second part contains seven chapters: Catalysis and enzymes; photosynthesis; the fats; nitrogen metabolism; ash; the transport of solutes; and respiration. The final part has four chapters: Germination; growth; reproduction; and irritability and plant movements. There is a brief appendix dealing with pH, a general bibliography, and author and subject indices. The total number of pages is 539, and the price not including the 15 per cent. advalorem duty, is 15 shillings. It is hoped that American plant physiologists will extend their acquaintance with the author by using it in connection with courses in plant physiology, and by making it available in libraries for the use of students. It contains a great deal of information, and reflects the points of view developed in the English laboratories, where many very valuable contributions have originated. The author deserves encouragement in his attempt to provide a text based upon the more fundamental general physiological processes. Orders may be sent to Williams and Norgate Ltd., Great Russell St., London. An American agent may be announced later.

Soilless Growth of Plants.—It was inevitable that the lay interest in tray agriculture, or solution culture of vegetables, flowers, and fruits, would be capitalized in book form. We record the publication of *Soilless Growth of Plants*, by the Reinhold Publishing Corporation, New York. The authors, Carleton Ellis and Miller W. Swaney, are not primarily plant physiologists, but private laboratory chemists (Ellis Laboratories, Montclair, New Jersey). The language is too "popular" in places, and misrepresents the points of view developed in plant physiological laboratories. This, perhaps, is to be expected, but the public gets a distorted idea from reading books that are not scrupulously accurate at the same time that they are popular reading. The reviewer believes that it is not necessary to depart from accuracy to make plant behavior interesting to the public.

This work is intended to educate the public in regard to the principles

and practices of solution culture plant production. And the public does need educating, as is revealed by the case of a man who approached the writer and proposed to sink a fortune of $25,000 in tray agriculture by using an old barn which could neither be heated nor lighted as the site of his operations! The difficulty is that chemical laboratories are selling salt combinations to a gullible fraction of the population, on the theory that soilless culture of plants is practicable for the ordinary individual.

There are eight chapters, as follows: Chemistry of plant life; growing in mineral aggregates; growing in water; household plant culture; commercial aspects; special chemicals; common detriments; and nutrient formulas.

It is written in entertaining style, and will no doubt have many readers. Those who contemplate using solution culture methods of growing plants should consult, at the same time, the agricultural experiment station bulletins which cover this subject with unvarnished accuracy. The statement by HOAGLAND and ARNON, issued in February 1938 by the University of California; Bulletin 636 of the New Jersey Station; and circular 232, of the Indiana Station are recommended as collateral reading. The News Letter of the Committee on the Relation of Electricity to Agriculture, number 17, June 1938, published at 58 East Washington St., Chicago, contains a series of papers from California, Wisconsin, Indiana, and New Jersey, explaining *Nutrient Solution Culture Methods* in an interesting manner.

The price of the ELLIS and SWANEY work is $2.75, and it can be ordered from the publishers, if not obtainable at local book stores.

General Physiology.—The third edition of Dr. PHILIP H. MITCHELL'S *General Physiology* has been issued by the McGraw-Hill Book Co. It has been extensively revised for this edition, especially with reference to excitation, contraction, chemical transmission, membrane structure, permeability, physiological oxidations, muscle chemistry, vitamins, hormones, etc. Intended for college students who may have but one course in physiology, the emphasis is largely upon animal physiology, although it is called general physiology. In many instances, however, the discussion applies just as well to plant cells as to animal cells, and is truly general. The reviewer considers it an excellent text for the group for which it has been prepared. Each of the 25 chapters closes with a literature list of monographs, reviews, and journal publications which serves as a guide to extensive reading. The work is worthy of attention by all who desire to keep abreast of the progress made in the field of general physiology. The quoted price is $6.00 per copy.

Laboratory Manual of General Physiology.—To accompany the text book of general physiology just noted, a laboratory manual has been prepared by Dr. P. H. MITCHELL and I. R. TAYLOR. The manual contains 254 exercises, arranged in 21 chapters, the order of which follows the order of

presentation of the text. The experiments are outlined in concise form, and offer a great variety of laboratory techniques. A good teacher should have no difficulty in providing an intensely interesting and valuable course from the suggested work. Many more experiments are outlined than can easily be performed during a single year, so selection will be necessary. The work can be adapted thus the more readily to individual laboratory equipment. The price of the manual (142 pp.) is $1.50 per copy.